Ecology and phylogeography

of an Australasian green turtle population:

a case study for conservation from Aru

Kiki Dethmers

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Aan mijn ouders

1.

General introduction

Introduction

The green turtle *Chelonia mydas* (Linneaeus, 1758) is an ancient living species that has survived since the Cretaceous, 145 million years ago, but in recent years, anthropogenic activities have caused a considerable decline in many populations worldwide. The species is, as a result, listed as globally endangered (IUCN Species Survival Commission 2007). While knowledge on the biology, status and fate of green turtle populations in the Atlantic and South Pacific has steadily increased over the past two decades, surprisingly little detailed information has been published from the Asian region. Many coastal indigenous peoples throughout this region have, for centuries, exploited this species for subsistence and cultural ceremonies. In recent times, subsistence harvesting of green turtles in many parts of Indonesia has expanded to include commercial harvesting as well, with a strong negative impact on population sizes.

In this thesis I describe my research into the relationships between green turtle populations in the Indo-Pacific region within the concept of a metapopulation. Metapopulations are assemblages of interacting populations with an expected time to extinction (Levins 1969), and are closely linked with the processes of population turnover, extinction, and establishment of new populations (Hanski & Gilpin 1991). The assemblage of green turtle populations in the Indo-Pacific region form a metapopulation under the assumption that these populations are more inter-related than they are related to populations in the Western Indian Ocean or in the Eastern Pacific. Under the metapopulation concept, it can thus be expected that local colonisation and extinction processes among the Indo-Pacific green turtle populations are in balance. The aim of this study is to create a better understanding of the susceptibility of these populations to local extinction. This thesis focuses on the green turtle population in the Aru archipelago in particular. This remote part of Indonesia supports one of the region's last remaining large populations of green turtles but this population has become subject to large-scale commercial exploitation activities. Situated on the frontier of SE Asia's heavily impacted marine ecosystems and biodiversity, and the comparatively well-managed marine resources in Australian waters, the situation in Aru presents a unique opportunity to conduct a comparative study of green turtle population dynamics and the effects of human activities on these dynamics. This thesis describes the distribution of, and relationships between, *C. mydas* populations within the Australasian region, and assesses the impact of subsistence and commercial exploitation on a single population in SE Indonesia as well as on related populations in the broader Australasian region.

Sea turtle life history

Marine turtles are an order within the class of Reptilia, with modified forelimbs adapted for swimming but poorly adapted for terrestrial locomotion, despite a commitment to terrestrial oviposition. The order of the Testudines is classified into two families (Brongniart 1805); the hardshelled Cheloniidae with six species representing five genera (*Chelonia mydas, Eretmochelys imbricata, Caretta caretta, Natator depressus, Lepidochelys olivacea* and *L. kempii*) and the leathery-shelled Dermochelyidae with one genus and one species (*Dermochelys coriacea*) (Pritchard 1997). All species, except *L. kempii* and *N. depressus* have a global distribution and all except *L. kempii* occur in Australasian waters.

The green turtle (*Chelonia mydas*) is a long-lived herbivore with complex life history traits that can vary substantially depending on the geographic location of a population (Figure 1.1 and table 1.1). The age at which females begin to reproduce varies between 20 – 50 years (e.g. Hirth 1997, Limpus & Chaloupka 1997) and detailed studies of nesting females in Australia found that, in this region the size at first reproduction is typically just below the average breeding size for the population (e.g. Limpus & Walter 1980). At the onset of the reproductive season, adults migrate from resident foraging habitat to their natal nesting beach. This migration may be over a thousand kilometres long (Carr & Ogren 1960, Meylan 1982, Limpus *et al.* 1992 and see table 1.1). Nesting periodicity or remigration interval ranges from 2 – 6 years (Carr & Carr 1972, Solow *et al.* 2002, Limpus *et al.* 2003) and the number of clutches deposited each season varies between 3 – 9 (Johnson & Ehrhart 1996, Bjorndal *et al.* 1999, Limpus *et al.* 2003), with a mean clutch size of 113 eggs (Miller 1997). Incubation time depends on temperature and ranges from 6 –13 weeks (Miller 1997), with temperature also affecting the sex of the progeny (Mrosovsky 1997). Once emerged from the nest, the life cycle goes through 3 distinct stages (Figure 1.1); pelagic hatchlings, developing immatures, and reproducing adults. Upon emergence of the nest the hatchlings instantly make for the sea with strong precision (Lohmann *et al.* 1997). After entering the water the hatchlings move towards the open ocean (Carr 1984, Frick 1976). The following life-stage is poorly understood but it is believed that the hatchlings rely on major ocean currents to be transported away from the natal beach to their pelagic nursery habitats (Carr 1987, Musick & Limpus 1997) and passively drift along ocean currents in association with *Sargassum* floats (Carr 1984). The next life-stage is characterized by the first recruitment from the pelagic phase to neritic developmental habitat, which generally occurs at sizes between 30 to 40 cm Curved Carapace Length (CCL) and age of approximately 5 years (Limpus & Chaloupka 1997). Along the east coast of Australia, females first start to breed at sizes ranging from 86 – 106.0 cm CCL (Limpus *et al.* 2003; Limpus *et al.* 2005). Survival is a key demographic component

of population growth and evolutionary fitness (Fox 1993). Mortality is highest during the early life stages, with an estimated hatchling survival rate between 40 – 48%, and an approximated mean survival rate of 65% for the pelagic stage for *C. mydas* in eastern Australia (Chaloupka 2002). Survival rates are highest in the mature adult stage, with a 93 – 97% chance of survival (Chaloupka & Limpus 2005). The protracted maturation stage and the difference in remigration intervals complicate estimation of population abundances (Hays 2000).

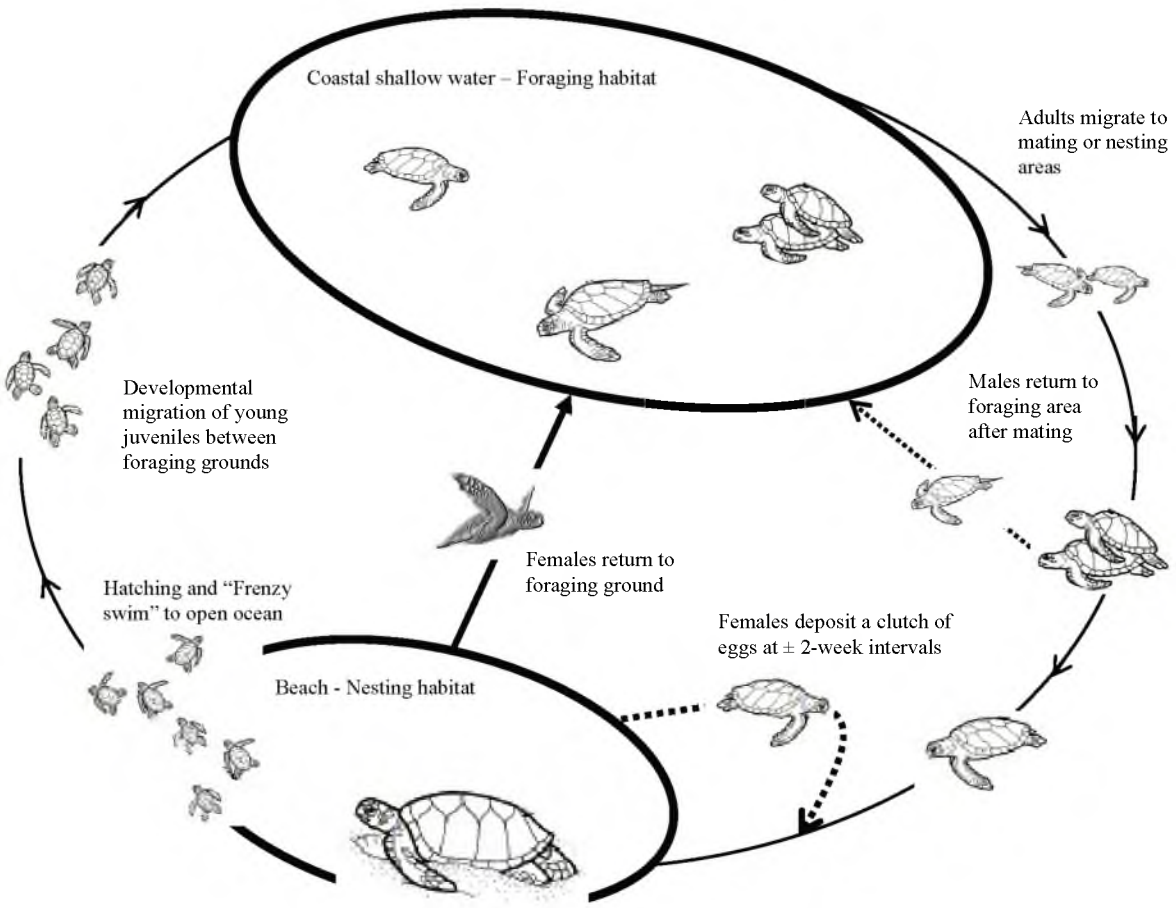


Figure 1.1. Life history cycle of the green turtle (*Chelonia mydas*). Adapted from Lanyon *et al.* 1989.

Table 1.1. Variation in life-history traits among global *Chelonia mydas* populations

Ocean	Population	Growth rate (cm/yr)	Remigration interval (years)	Migration distance (km)	Source
Pacific	sGBR	3.8			Limpus & Chaloupka 1997
Ocean	nGBR		5.5		Limpus *et al.* 2003
	Hawaii	5.7	3.5	1073	Balazs 1994; Balazs & Chaloupka 2004a; Balazs & Chaloupka 2004b
	Japan	6.8			Chaloupka *et al.* 2008
	WanAn			687	Cheng 2000
Atlantic	Costa Rica	4.9	2.5	512	Hirth & Carr 1970; Troeng *et al.* 2005; Chaloupka *et al.* 2008
Ocean	Florida	13.9			Bjorndal *et al.* 2000
	Ascension		3.5	1968	Mortimer & Carr 1987; Luschi *et al.* 1998
	Cyprus			1076	Godley *et al.* 2002

Sea turtles in Indonesia

In Indonesia, marine turtle products have been traded and exported for many centuries. In recent history, a variety of products including tortoiseshell articles, stuffed turtles, and meat were traded at domestic markets and exported to Japan, Singapore, and to a lesser extent to other destinations in Europe and USA (Polunin 1975; Groombridge & Luxmoore 1987). In the late sixties and early seventies, Indonesia exported an estimated 25.000 – 50,000 stuffed turtles (green turtles, *Chelonia mydas*, and hawksbill turtles, *Eretmochelys imbricata*) annually. Despite Indonesia's ratification in 1978 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), exports continued until at least 1990 (Groombridge & Luxmoore 1987, Milleken 1990). Toward the end of the 20th century, exploitation of turtles for meat was estimated to be about 25,000 *C. mydas* per year, most of which were consumed in Bali as part of Hindu traditional and social ceremonies. In addition, intensive egg harvests occurred at virtually all nesting beaches throughout the Archipelago, showing evidence of overexploitation and subsequent population declines at three exploited nesting beaches (Sloan *et al.* 1994, Wicaksono 1992, Polunin 1975, Limpus 1997).

Decades of heavy exploitation and obvious declines in abundance of the turtles have led to grim predictions for the outlook of Indonesian turtle populations. The 54,500 km of coastline and 17,000 islands support a large range of nesting and foraging habitat for five sea turtle species (Figure 1.2), with *C. mydas* as the most commonly encountered (Halim & Dermawan 1999). However, despite its widespread distribution in this region, very little is known about population structure, dispersal, demography and reproductive biology of *C. mydas*. Worldwide, population declines have been attributed to, among other things, i) loss of critical nesting habitat as a result of human encroachment, ii) loss of feeding habitat through destructive fishing practices, iii) by-catch in trawl fisheries and iv) entanglement in discarded drift nets. In Indonesia, however, it is the unrestricted harvesting of turtles and eggs which has raised concerns for the conservation status of marine turtles. One of the main problems has been a lack of systematic information and availability of baseline data for determining trends.

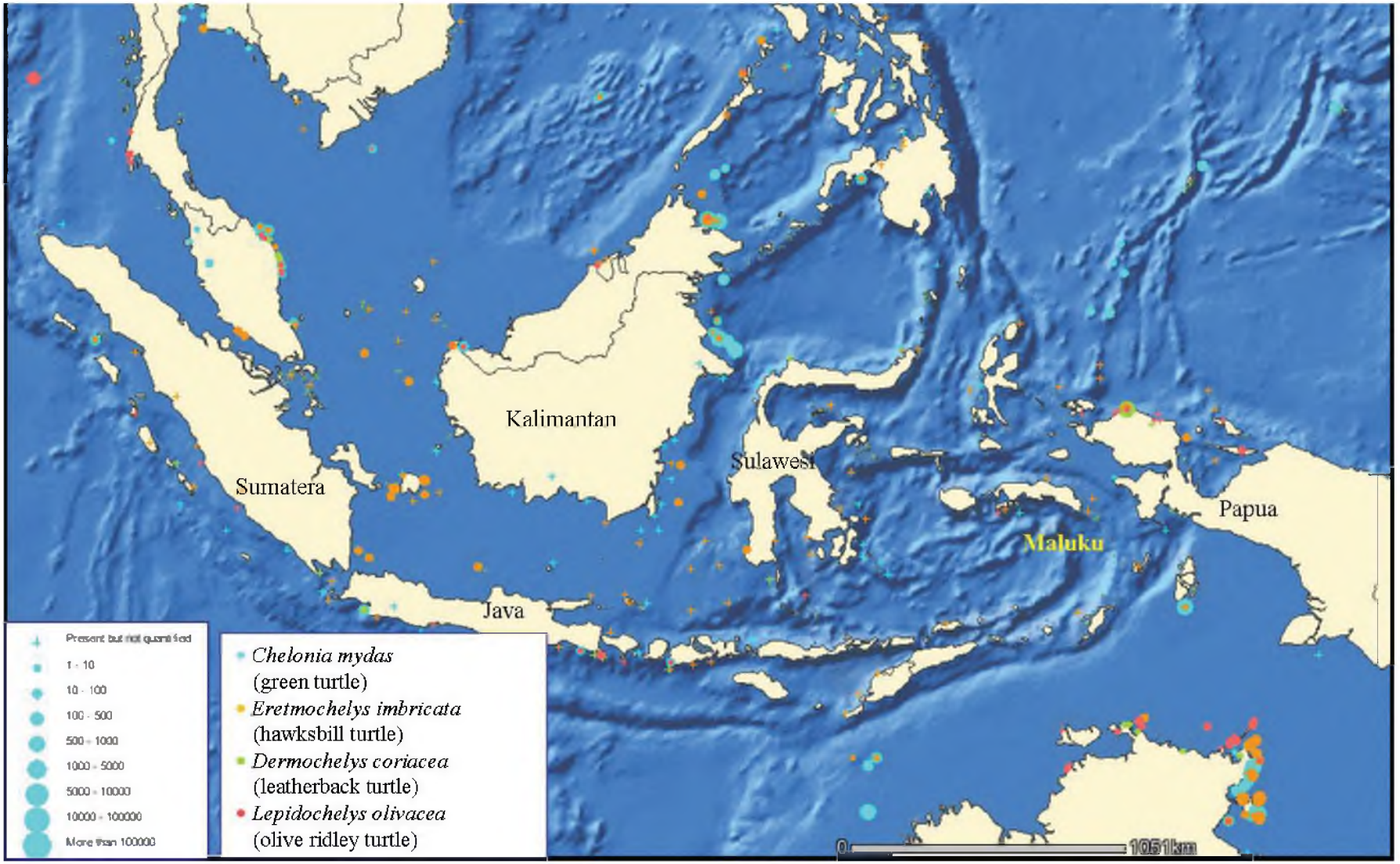


Figure 1.2. Distribution and estimated size of marine turtle nesting populations in the Australasian region. Rookery size data have been derived from the Marine Turtle Interactive Mapping System (UNEP/CMS www.unep-wcmc.org) and the Marine Turtle Database maintained by C. J. Limpus at Queensland Parks and Wildlife Service.

In 1980, a survey on trade of marine resources in the far Southeast Indonesian archipelago of Aru, revealed a significant nesting population of *C. mydas* (Compost 1980). Subsequent investigations in this region (unpublished reports 1989, 1992, 1993, and Schulz 1996) found that substantial numbers of adult turtles were harvested and shipped out to regions outside Aru. The exploitation occurred both at the nesting and foraging habitat. This remote part of Indonesia supports one of the region's last remaining large populations of green turtles but its persistence is threatened by large-scale exploitation activities.

Aru

The Aru archipelago is located in the southeast of the province Maluku and its natural richness was first described by Alfred Russell Wallace (1869) 150 years ago. The shallow coastal waters to the southeast of Aru have an exceptionally high biological diversity and have been considered among the richest marine areas in Indonesia (Compost 1980). Seagrass beds and coral reefs along the north and east coast provide forage and habitat for formerly large numbers of green and hawksbill turtles (*Eretmochelys imbricata*), as well as dugong and diverse assemblages of fishes, sharks, rays, and invertebrates, including many commercially important species. Historically, both green and hawksbill turtles frequented the islands Enu, Jeh and Karang to nest and this area was believed to hold one of the largest green turtle nesting areas (also referred to as rookeries) in Indonesia (Compost 1980). During the past decade, only the 10 km of sandy beach on Enu island is still visited by large numbers of green turtles, possibly as a result of two decades of intensive harvesting practices. In 1991, an area of 114.000 ha was declared as the Aru Tenggara Marine Reserve (ATMR) by governmental decree (Pemerintah RI 1999), encompassing six uninhabited islands (Figure 1.3). While a seemingly good step, the decree has several serious flaws. For example, the area declared to be a strict marine reserve is not demarcated, and as a result it is unclear where protective regulations associated with the status of "strict reserve" apply. Furthermore, the decree states that various marine species such as the dugong (*Dugong dugon*), turtles (*Dermochelys coriacea* and *Caretta caretta*) and the saltwater crocodile (*Crocodylus porosus*) are protected, as well as their habitat. Ironically, neither leatherback nor loggerhead turtles have been observed in the reserve or its surroundings. Species that do occur in the area, such as the green and hawksbill turtles, are not listed in the decree.

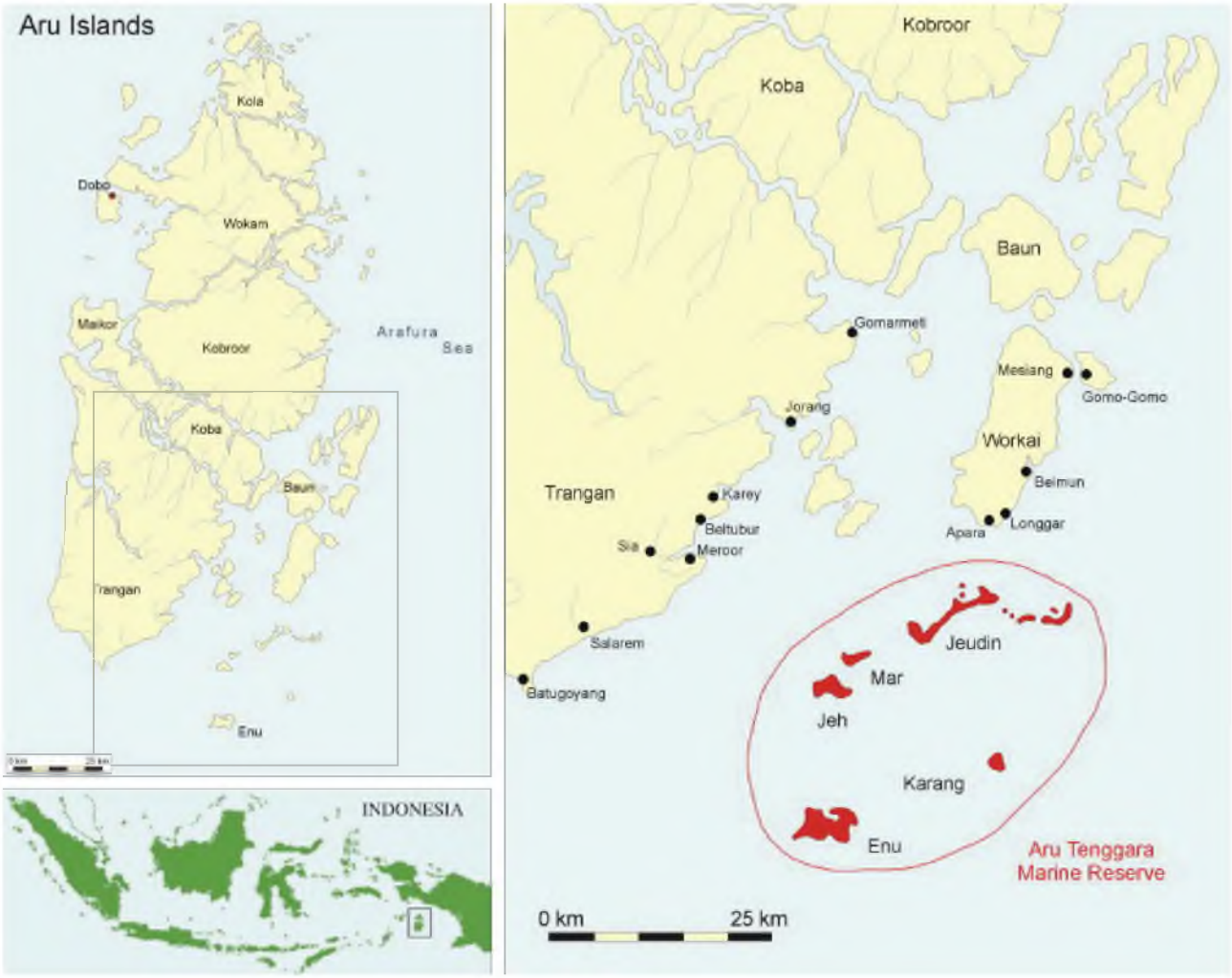


Figure 1.3. Geographic location of the Aru archipelago, the main islands where *Chelonia mydas* comes ashore to nest (in red), and the approximate contours of the Aru Tenggara Marine Reserve.

Exploitation

Subsistence exploitation

Aru's approximately 63,000 inhabitants are fully dependent on marine resources, as no agriculture or any major form of industry occurs. In Aru, as in many parts of the Indo-Pacific region, turtles and their eggs have been subject to subsistence harvests for centuries. With nesting beaches and foraging grounds located in the eastern areas of the archipelago, consumption of turtle meat and eggs is primarily restricted to coastal communities in the east. All the nesting beaches are located at least half a day's travel away from any coastal community, thus to collect turtles or eggs requires substantial effort in terms of travel time and costs. The harvest of turtles and eggs from a nesting beach is generally done in

conjunction with, or as an extension of, fishing journeys or excursions made to collect sea cucumber (trepang; Holothuroidea). Occasionally, the nesting turtles are targeted in preparation for weddings, harvest celebrations or religious festivities organised by the Protestant and Catholic members of the communities. The size of a catch is generally restricted by the size of the vessels and could be between two to ten turtles. Exploitation of turtles at the foraging grounds is more targeted, as the travel distance to the feeding grounds is shorter than to any of the nesting beaches. The animals are caught with harpoons at the feeding grounds. Contrary to neighbouring societies in the region such as the Caroline islands (McCoy 1974), the Tokelauans (Johannes 1978), Torres Strait Islands (Johannes & MacFarlane 1991), aboriginal communities in the Gulf of Carpentaria (Kennett *et al.* 1998), or at the nearby Kei islands (Barraud 1990, Suarez & Starbird 1996), the Arunese have no traditional ceremonies which necessarily involve the consumption of turtle meat (Osseweijer 2001). A small local trade of turtle meat and products exists and is aimed at the main township of Dobo, on the west coast, which usually takes place in conjunction with other business trips into the town.

Commercial exploitation

The relatively opportunistic nature of the local harvest and the sparse human population along the east coast of Aru most probably did not pose an immediate threat to the persistence of the turtle population in this area. However, starting in the late 1970s, large Buginese schooners from Makassar arrived in Aru to collect turtles for the market in Bali. As indicated previously, Bali has long been known as one of the biggest markets for turtle meat worldwide with annual landings of 10 to 30 thousand turtles (Salm 1984 and WWF pers. comm.). The Balinese predominantly adhere to Hinduism and traditional religious ceremonies require the use of turtle meat, though Hindu high priests have estimated that only 300 to 500 turtles annually would serve that purpose (WWF pers. comm.). Thus, the majority of the turtles landed in the harbour of Tanjung Benoa are destined for the domestic market. To meet an increasing demand, the Bali turtle trade expanded in the 1980's to incorporate distant feeding and nesting populations throughout the entire Indonesian archipelago, with significant annual takes in distant waters such as the Arafura sea surrounding the Aru archipelago (Schulz 1984, 1989).

In his report on a survey of exploitation of dugong and sea turtle in the Aru, Compost (1980) expresses his concern regarding the magnitude of this commercially driven exploitation and its potential impact on the Aru turtle population. In addition to an off-take at the nesting beaches, turtles were also caught in large numbers off the feeding grounds and an estimated 300 thousand to 2 million eggs were taken from the beaches every year (Compost 1980, Schulz 1996). Subsequently, a decrease in nesting females was observed over the following

years. Schulz estimated 3000 females nested on Enu Island in 1988 (Schulz 1989), 50% less than the estimate made by Compost (1980), and in 1993 this number was estimated at 1000 females (Schulz 1993, Sahertian & Noija 1994). These figures seem to suggest that the population of nesting females in SE Aru has experienced a serious decline over a period of approximately 15 years. Green turtles migrate between nesting and feeding habitat and studies have shown that Indonesia shares its turtles with neighbouring countries. For example, turtles tagged at Australian nesting beaches have been found to forage at Indonesian sea grass areas (Limpus *et al.* 1992). It is therefore likely that green turtle populations nesting in areas outside of Aru may be affected by the exploitation activities in SE Indonesia.

Ecological role of sea turtles

Current sea turtle abundance is believed to be only a fraction of its historical (14th century) population sizes (Bjorndal & Jackson 2003). As a result of the massive declines, the past roles of sea turtles as major marine consumers are no longer apparent and this species' influence on its environment has been forgotten. Therefore, there exists no reliable baseline for sea turtles against which to assess the wider effects of the population declines. An understanding the ecological role of sea turtles contributes to a better understanding of the characteristics of ecosystem functioning. Several studies have demonstrated that removing consumers from marine ecosystems has far-reaching effects (as summarized in Bjorndal and Jackson 2003). Seagrass is the primary diet of green turtles (Bjorndal 1997). Major changes in a grazing regime can be expected to result in major changes in biodiversity, productivity and structure of seagrass beds. For example, ungrazed seagrass beds shorten nutrient cycling times reducing primary productivity (Thayer *et al.* 1984); increase particle entrapment and substrate depositions, altering the physical structure of important fish-nurseries (Jackson *et al.* 2001); and increase the chance of sulphide toxicity and overgrowth by epibionts (Harvell 1999). Thus, massive reductions in the abundance of turtles in Aru can be expected to have a far-reaching impact on the seagrass beds and the associated marine biodiversity, both from an ecological as well as from an economical perspective. Understanding of the biological mechanisms that drive the distribution and abundance of an exploited species will be essential if conservation efforts are to be converted into conservation successes. With this in mind, this study focuses on compiling the basic population dynamic parameters, establishing links in space and variation in time to describe the impact of exploitation on green turtles in Aru and the wider Southeast Asian region.

Outline of this thesis

The aim of this research is to gain an understanding of the structure of the Indo-Pacific green turtle meta-population in general and the green turtle population in Aru in particular and obtain insight into the effects of large-scale commercial exploitation of this species. This study intends to present a backbone against which conservation strategies can be drafted and management decisions can be made. **Chapter two** presents a detailed description of the green turtle nesting population of Aru and provides the essential demographic data needed for population persistence and metapopulation studies. The data for this chapter were obtained during four consecutive census periods from 1997 to 2000, as well as from an in-situ experiment on the influence of nesting substrate type on hatching and emergence success. Genetic analyses using mtDNA variation in combination with mark-recapture data is used in **chapter three** to assess the geographic range occupied by individual breeding populations and the distribution of such populations through Australasia. Assessments of mtDNA haplotype variation among 27 green turtle rookeries is used to try to reveal the level at which adult female disperse among nesting sites in the region. Genetic tests of population differentiation of these rookeries have been used to characterise distinct breeding populations or groups of populations (referred to as breeding stocks). In **chapter four** the same genetic tests were used to assess migratory connectivity between feeding grounds and the breeding stocks. Special attention is given to the migratory pattern of the Aru stock that migrates away from the Aru feeding grounds to test the hypothesis that this stock is for the most part sedentary in that it breeds and forages in the same region, and therefore is likely to be more vulnerable under the pressure of the commercial harvest. In **chapter five** an age-based stochastic population model is used to assess the impact of the observed harvest on persistence of the Aru stock and investigate the effect of several management approaches aimed to reduce the probability of extirpation. A more detailed discussion of various forms of green turtle exploitation in Aru and the conservation status of the Aru population in the context of the Aru Tenggara marine reserve is presented in **chapter six**. The major findings of this study are presented in the synopsis in **the summary chapter.**

2.

Population structure and reproductive biology of green turtles (*Chelonia mydas*) nesting in Aru, SE Indonesia

Dethmers K., van Groenendael J., Nienhuis P.

Manuscript

Abstract

Indonesia's geographically wide distribution of about 17,000 islands, contains 100 known green turtle (*Chelonia mydas*) nesting areas. Surprisingly, however, few quantitative studies exist on this species' population structure, demography and reproductive biology in Indonesia. Many *C. mydas* populations throughout Indonesia have declined as a result of overexploitation or other anthropogenic and environmental sources of mortality. This study compiles comprehensive population demographic information on a large population of *C. mydas* nesting in the Aru Islands (Southwest Moluccas), providing essential data for population persistence and metapopulation dynamics studies. Beach-patrols were carried out across four nesting seasons from 1997 to 2001 to quantify the number of female nesting attempts, nest-densities, and hatching success, as well as the temporal and spatial variation in nesting density. An in-situ experiment tested the influence of the nesting substrate type on hatching and emergence success. *C. mydas* nests year-round in Aru, with a distinct peak between November and March. The annual nesting population size of C. *mydas* in Aru falls within the size-class of 500 – 1000 individuals, with fluctuating population densities across years. Only a small proportion (23%) of all nesting attempts in the 1997/1998 season were successful, with only 1.3 ± 0.03 clutches per female.

Introduction

The green turtle (*Chelonia mydas*, (Linnaeus 1758)) is one of seven existing marine turtle species subdivided into two families, Cheloniidae and Dermochelyidae that belong to the order of Testudines. All but one species occur in Indonesian waters, and all species share common features and life history traits, such as: large and multiple clutches of eggs per nesting season, late sexual maturity with low survival rate till sexual maturity (Hirth 1997), and high fidelity to nesting and foraging habitat.

C. mydas has a circumglobal distribution, occurring throughout tropical and, to a lesser extent, subtropical waters. The global population is separated by strong genetic divergence between population nesting in the Atlantic and Pacific regions (Bowen *et al.* 1992), and nesting occurs in more than 80 countries worldwide (Hirth 1997). The global population of *C. mydas* could thus be viewed as two metapopulations within which local populations are connected by dispersing individuals. These metapopulations might differ in terms of ecology and life-history traits. Understanding the dynamics of a metapopulation is crucial for formulating more precise predictions on the probability of extinction under various management scenarios. Compared to the Atlantic populations, relatively little quantitative information exists on *C. mydas* populations in the Indo-Pacific region, with the exception of populations in Australia. Within Indonesia, there are approximately one hundred known *C. mydas* nesting areas (UNEP/CMS www.unep-wcmc.org). No quantitative data on population densities exist at 90% of these nesting areas, while at most of the remaining 10% of nesting areas, densities are believed to be no more than 100 nesting individuals annually. Only four nesting areas are known to host more than several hundred (and indeed, up to several thousand) nesting *C. mydas* every year; Aceh (north Sumatra), Pangumbahan (west Java), Berau Islands (east Kalimantan), and the Aru Islands (southwest Moluccas). Despite its widespread distribution in the Indonesian region, very little is known about the population structure, demography and reproductive biology of Indonesian *C. mydas*, and thus how the Indonesian populations contribute to the dynamics of the larger Indo-Pacific metapopulation.

Marine turtles are, as a result of the predominantly aquatic phase, logistically difficult to study, and are therefore best studied when females come ashore to lay eggs. As in all species of marine turtles, *C. mydas* exhibits iteroperous reproduction. Typically, the turtles nest on exposed marine beaches, in deep, clean and relatively loose sand above the high-tide mark; usually at night (Hendrickson 1958). What follows is stereotyped nesting behaviour, which involves an approach to the beach, ascent of the beach, wandering on the high beach, digging a body pit, digging an egg chamber, laying of eggs, covering the nest, and return to the sea

(Hendrickson 1958). On average, one hundred eggs are deposited during up to ten nesting events that occur at regular intervals of approximately two weeks over the course of the reproductive period. There is strong philopatry to a specific nesting location, but intra-specific spatial and temporal variation exists in the extent of this philopatry. After emerging from the nest, all hatchlings move immediately to the sea, usually after dark. While this behaviour seems to be consistent among populations worldwide, differences have been observed between populations along the east coast of Australia (Pacific) and Costa Rica (Atlantic) in, for example, the average number of nests per female (6.2 and 2.8 respectively) and the mean interval between two consecutive nesting seasons per individual (5.5 and 2.5 years respectively, Limpus *et al.* 2001; Limpus *et al.* 2003; Bjorndal *et al.* 1999; Troeng & Rankin 2005). Little detailed documentation exists of nesting behaviour and nesting success of Indonesian *C. mydas* populations.

The potential significance of the *C. mydas* population in Aru was first established in 1980, when a pilot survey investigated the occurrence, number, and trade of sea turtle species and dugong (*Dugong dugon*) in the Aru archipelago (Compost 1980). A group of several small islands in the Southeast of the archipelago constitute the main nesting areas. In subsequent surveys, Schulz and his team estimated that 3 to 4 thousand turtles nest on the largest island, Enu, each year (unpublished reports Schulz 1989, 1992, 1993, and 1996). Based on these estimates, Enu Island was considered the most important nesting area for *C. mydas* in East Indonesia. However, investigations of local turtle exploitation rates estimated that 300 thousand to 2 million eggs were taken from the beaches, as well as 3 to 6 thousand turtles taken from the nesting and foraging habitat in Aru every year (Compost 1980, Schulz 1996). Whether the population can persist under such exploitation pressure can only be reliably estimated with comprehensive population demographic information.

The majority of *C. mydas* populations throughout Indonesia and most other parts of the Australasian region have suffered significant population declines (summarised in Limpus 1997). Most reported studies have attributed such declines to overexploitation of the adult turtles or their eggs. However, with the exception of egg-harvest concession data from Pangumbahan, southwest Java (Sloan *et al.* 1994) and Berau, east Kalimantan (Wicaksono 1992), there are no long term census data of nesting *C. mydas* from any beach that is suitable for assessing long term population trends. It is thus, critical that such information is collected so that firm conclusions on regional *C. mydas* population status are possible.

The main aim of this study is to compile comprehensive demographic data of the *C. mydas* population that nests in SE Aru, which will form the basis for future population persistence

studies in this area as well as in the broader Australasian region. Our study documents the spatial and temporal distribution of nesting activity on the main nesting beach of Enu Island and focuses specifically on identifying the occurrence and length of a peak in the nesting season. In addition, we estimate the annual nesting population size, assess nesting success, and analyse hatching success from different substrate types used for nesting to obtain a measure for reproductive success.

Methods

Study site

The Aru archipelago is one of the most remote areas of Indonesia and is situated on the edge of the Sahul shelf (Figure 2.1). This shelf once connected Aru with the Australian continent and New Guinea until rising sea levels submerged the plateau. Current sea levels were reached 6000 years ago (Chappell & Shackleton 1986). The shallow platform surrounding the eastern islands consists of a mosaic of seagrass beds, fringing coral reefs and tidal flats, constituting feeding habitat for primarily green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles. Olive ridley (*Lepidochelys olivacea*) and flatback (*Natator depressus*) turtles have also been observed occasionally by fishermen, though never on the beaches.

The beaches of the islands Enu, Karang, Jeh, and Mar in the far southeast of the Aru archipelago provide nesting habitat for green and hawksbill turtles. The largest island, Enu, located at 7°05'S and 134°30'E, is the southern most island of the Aru archipelago (Figure 2.1). It measures approximately 17 km in circumference, at its widest it measures 5.6 km west to east and 3.4 km south to north and with its total of seven kilometres of beach it constitutes the most important nesting site for *C. mydas*.

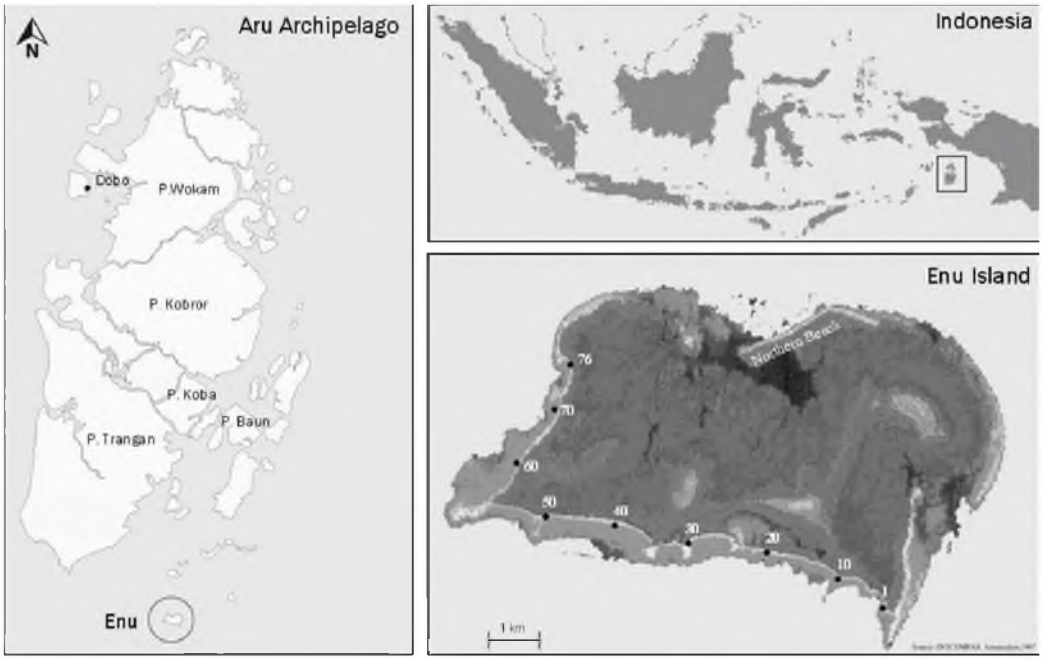


Figure 2.1. Geographic location of Enu Island, the main nesting habitat for green turtles (*Chelonia mydas*) in southeast Aru, with the main beaches along the western and southern coasts. Numbers refer to 1000-metre sectors on the beach.

The climate in Aru is strongly affected by monsoon and trade winds that influence nesting activities as well as human presence on the beaches. The wet northwest monsoon winds start in December and bring heavy storms and rainfall from January to late March. Onshore winds and large swells from the northwest make it extremely hazardous to land on the western beaches of Enu Island. As a result, the northwest monsoon is the time of the year when generally very few people visit any of the south-eastern islands and thus egg collection and the take of turtles off the beach is considerably reduced. Starting in May, the climate is controlled by dry south-easterly continental trade winds that blow from the cooler Australian mass and lasts from May to late September. Dry winds, clear skies and the largest variation in diurnal temperatures dominate this time of the year, with the strongest winds in July and August. The dry condition of the beach substrate thwarts the digging of a nest chamber as the dry sand easily caves in. Extreme dry conditions occurred in the year 1997, a severe El Niño year. November and April are typical transition months, when winds and weather patterns are unsettled. During the southeast monsoon, the western beach provides shelter for many large and small fishing vessels and the crew goes on-shore primarily to collect turtle eggs or adult nesting females.

Tidal wave propagation from the Pacific and Indian oceans across the shelves and into the oceanic basins cause the tides of the Indonesian seas to co-oscillate (Tomascik *et al.* 1997). In combination with a complex bathymetry and coastal geometry, the tides around Aru have a mixed to semidiurnal pattern, with two highs and two lows each day but of unequal elevation and time of high water (Figure 2.2).

Winds, currents and tide have a strong influence on the morphology of the nesting habitat and nesting substrate. The western beach of Enu Island is a moderately exposed monsoon beach, with a narrow sandy beach. The southern beaches constitute a typical coral coastline, producing coral debris and coral sand. Heavy storms and associated wave impact during the southeast monsoon can result in substantial beach abrasion, which hampers access for turtles emerging at this side of the island (Figure 2.3 a).

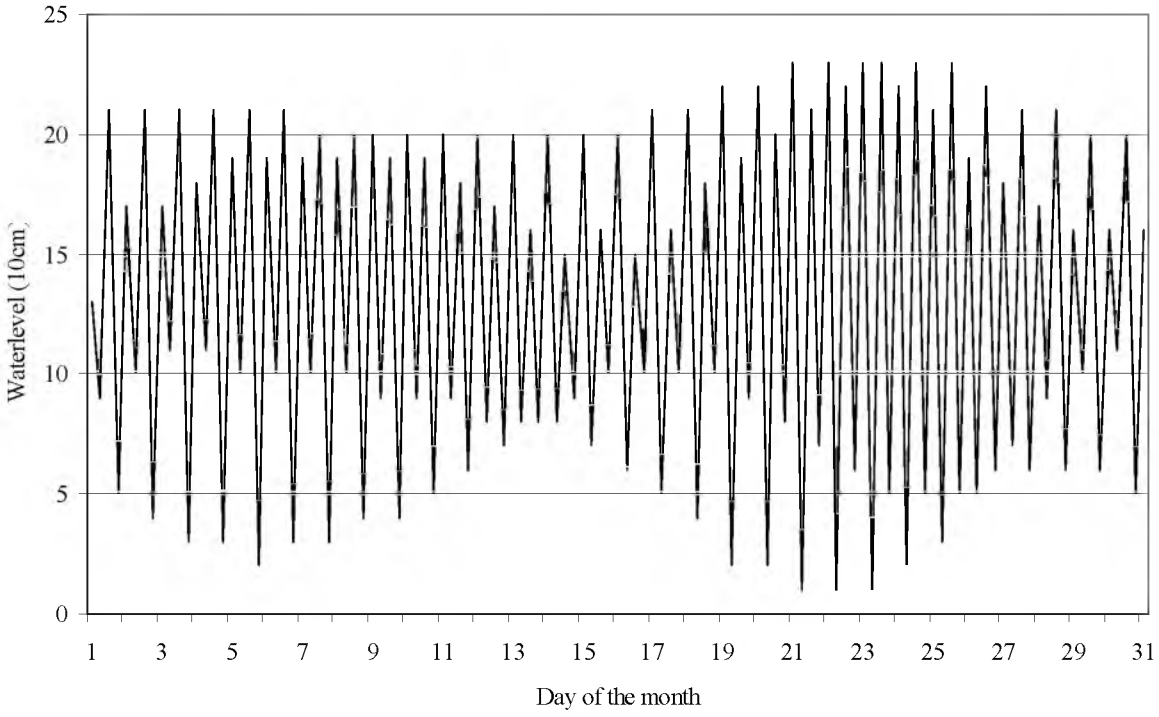


Figure 2.2. Estimated tidal movements as published by the Hydro-oceanographic bureau for Dobo, Aru in July 1997. (Source: Dinas Hidro-Oseanografi, Tide Tables Indonesian Archipelago 1997)

a

b

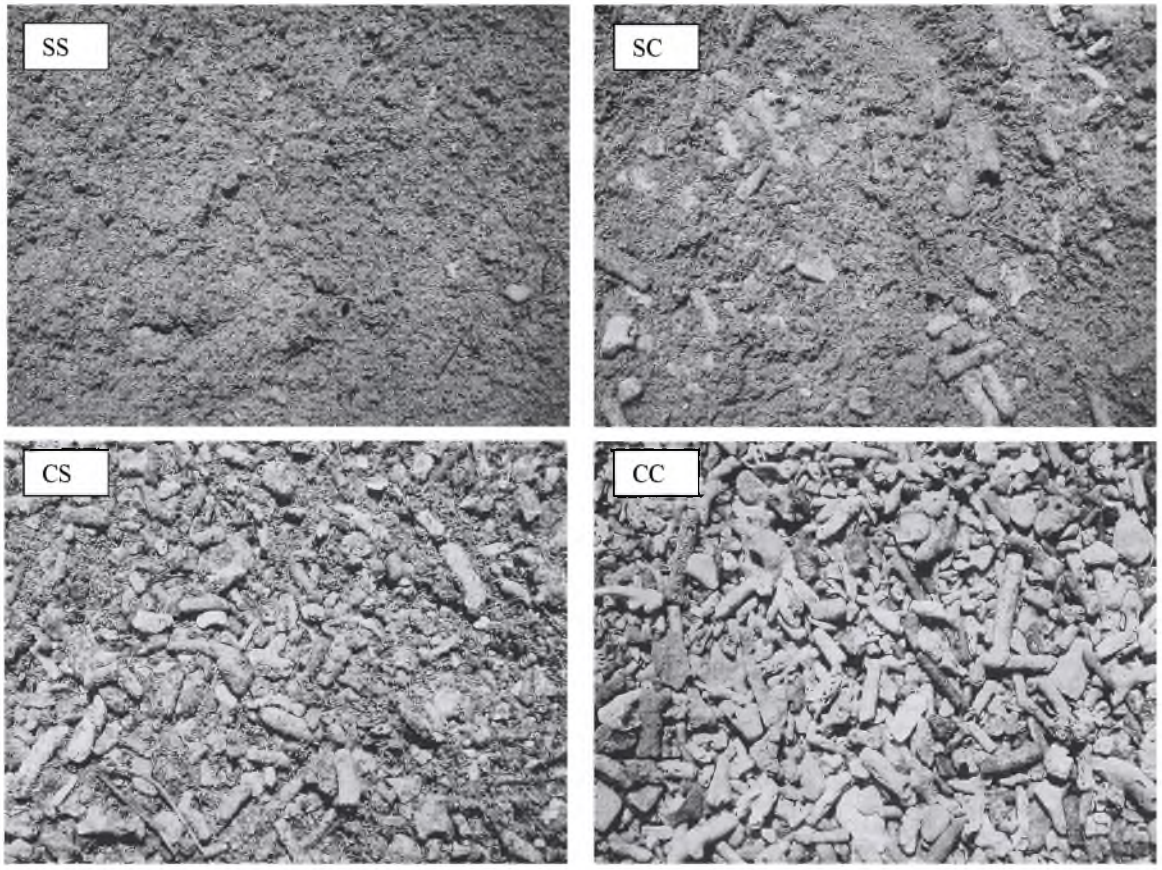


Figure 2.3. Beach morphology and abrasion, resulting in difficulties for turtles to access suitable nesting habitat or to dig a nest (a), and substrate types for hatching experiment (b). SS = 100% sand, SC = 75% sand and 25% coral, CS = 25% sand and 76% coral and CC = 100 % coral (images of experimental fields by D. Bezdickova).

Surveys

Surveys were carried out across four nesting seasons which run from July to June. The census periods were selected to cover an entire nesting season (1997/1998) as well as a fixed period across several seasons (November and April). As a result of the difficult climatic circumstances, no surveys were carried out from December to mid-March.

The main nesting beaches along the southern and western coastline of Enu Island were divided into marked sectors of 100 metres in length (Figure 2.1). At the start of each survey period all turtle tracks already present were counted and marked to avoid being recounted. Each night a team of two people patrolled the west and south beach on foot, starting one hour before sun-set during periods of early high tides, or three hours before late high tide to locate nesting turtles. At daylight the beaches were patrolled again to check for late emergences of nesting females. The location and fate of each track was noted and subsequently erased to prevent double counting. An estimate of the size of the 1997/1998 nesting cohort was established based on a combination of numbers of tracks per night, number of nests, and on tagged individuals (see below).

Counts and measurements

All turtles that came on shore were assumed to be sexually mature because no turtles were observed to come on shore for reasons other than to nest. Each track was, thus considered a nesting attempt. Successful nesting attempts were identified either by observing an individual depositing a clutch of eggs or, if no turtle was seen associated with a track, by examining the fate of that track. The fate of each observed track was identified by following the direction of the track as the individual ascended the beach. If the track returned to the sea without the sand-disturbance that is typical for a completed nest (Miller 1997), the nesting attempt was considered unsuccessful. If such sand-disturbance was observed, the attempt was marked as 'successful but no turtle observed'. If some other form of sand disturbance was observed which did not clearly identify the presence of a nest but could have indicated some form of digging activity, the attempt was marked as undetermined. Substrate type at each observed nesting attempt was recorded as 100% sand, mostly sand mixed with some coral rubble, mostly coral rubble mixed with some sand, or 100% coral rubble (Figure 2.3 b).

Identification of basic reproductive parameters such as size class, and reproductive state are prerequisites for most studies on reproductive cycles. The size range of mature females on Enu Island is based on measurements of body size of adult females nesting on Enu Island. To correspond with most major studies on sea turtles in Australia (Limpus *et al.* 1983), the animals were measured across the length and curve of the carapace (CCL). CCL

measurements were taken with a tape measure stretched tightly from edge to edge along the midline from the anterior skin-carapace junction to the apex of the posterior midline notch between the supracaudal scutes.

Clutch size and emergence success was assessed by excavation of the nest after hatching. The leathery texture of turtle eggs make that the shells remain in one piece after the hatchling has emerged and thus present a reliable way to measure clutch size and number of hatched eggs. Date, time, sector, observer's and recorder's names, and possible additional comments were noted for each observed nesting attempt.

Tagging

Upon completion of a nesting attempt, each turtle was marked with two standard, self-locking monel tags, ordered from National Band and Tag Company, Kentucky; size 1005, style 56, engraved with numbers in a series from X5001 - X6001 on the front, and on the reverse with the following return address; PUSDI – PSL – UNPATTI, Jl. M. Alfons. Poka. Ambon 97233. Indonesia. The tags were applied to the proximal rear edge of both front flippers, between the first and second large scales immediately adjacent to the axilla, following Limpus (1992). Before new tags were applied each turtle was checked for old tag scars. If on subsequent recaptures one of the two tags was missing, a new tag was applied to replace it, ensuring each turtle always carried 2 tags. This allowed identification and tracking of individual turtles through the course of the nesting seasons and across the island. A total of 623 individual turtles were tagged throughout the census period, of which 564 during the 1997/1998 nesting season and 59 individuals during the November 1998 and April 2000 surveys.

Hatching and emergence experiment

The influence of the nesting substrate type on hatching and mergence success was studied in a field experiment. Four experimental fields were created with nesting substrates of known composition reflecting comparable local conditions, including 100% sand (SS), 3:1 sand-coral ratio (SC), 1:3 sand-coral ratio (CS) and 100% coral (Figure 2.3b). Donor eggs were collected from 6 clutches (by 6 individual turtles) in the vicinity of the experimental fields so as to reduce the impact of egg translocation. Eggs were collected immediately after deposition of a clutch. From each clutch, 80 eggs were selected randomly and divided into four equal groups of 20 eggs. The experiment had 6 replicates. The eggs were carefully buried into the experimental fields at a depth of 40 cm. The remaining eggs in the clutch were left to develop in the 'donor' nest. Handling of the eggs was finished within four hours after oviposition to minimize handling induced mortality (Limpus *et al.* 1979). The experimental fields were protected against natural predators. Incubation period, hatching success (number of hatched

eggs / total clutch size), emergence success (number of self emerged hatchlings / number of hatched eggs) and dead hatchlings were recorded. Emerging hatchlings were measured for straight and curved carapace lengths, weight and length of front and rear flippers. The nests were excavated only if a) all 20 hatchlings had emerged, b) if less than 20, three days after the last hatchling had emerged, and c) if the first hatchlings originally belonging to the same clutch emerged more than seven days earlier in other substrates. Incubation, hatching and emergence results from the experimental nests were compared to those from the donor nests, as well as to those from nests not involved in the experiment.

Statistical analysis

All data sets were analysed in SPSS 11.5.0 (SPSS Inc. 2002). Comparisons of independent observations were analysed with Chi-square tests, and when significantly different, more detailed analyses were done using Mann-Whitney-U tests (Confidence Interval $\alpha = 0.05$). For multiple comparisons, the significance level was Bonferroni corrected.

Results

In the 1997/1998 nesting season recruitment to the rookery, as measured by the number of primary tags applied (n = 564), increased from July to November, peaked in April and by the end of the census period, only 20 new turtles arrived to commence their breeding cycle (Figure 2.4).

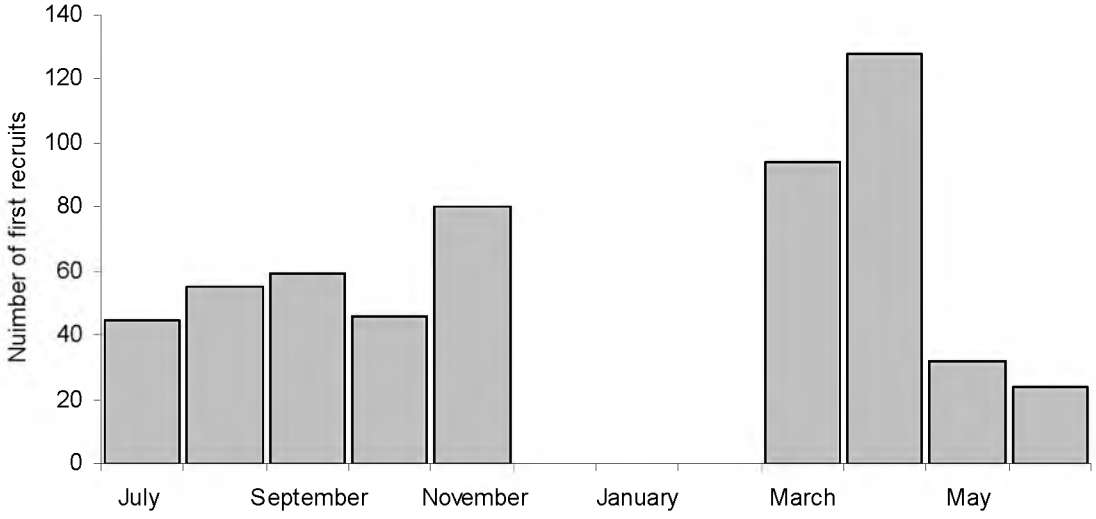


Figure 2.4. Recruitment to the Enu rookery across the 1997/1998 breeding season, measured as the number of primary tags applied in each month. No surveys were carried out in

December and January, and no tags applied during the one-day survey in February (see Figure 2.5a)

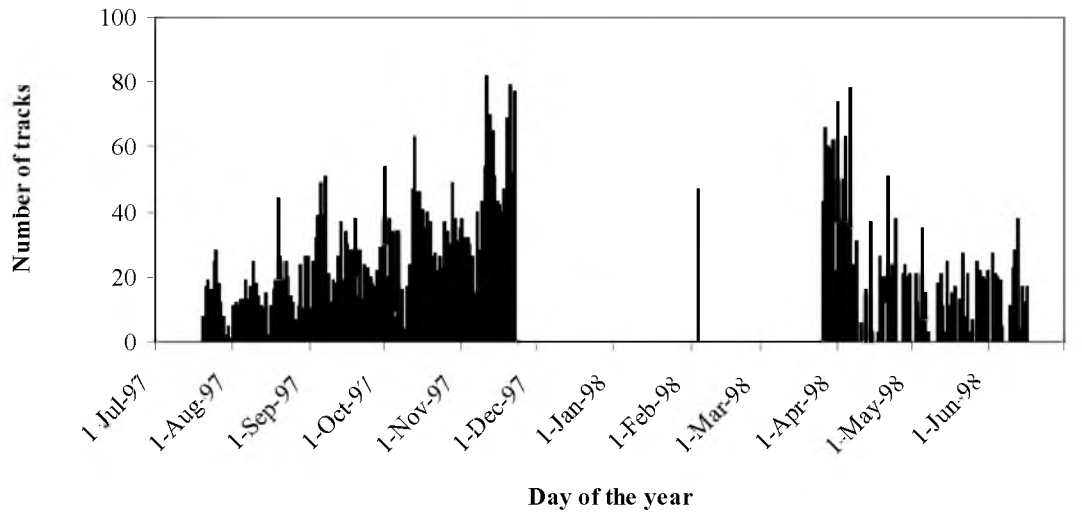
All nesting attempts occurred at night. Nesting occurs year-round with several peaks between November and April (Figure 2.5a). Although no data were collected during most of the peak of the west monsoon (December – February), a one-day survey on February 4th 1997 found 50 fresh tracks and many old tracks. This provided evidence for abundant nesting attempts in that month. More tracks were counted earlier in the season (n = 3493), i.e. from July to November than in the second half of the season (March – June: 1732).

The tagged individuals accounted for 1377 of the total of 5225 tracks observed during this season, resulting in 24.4% sighting rate of all tracks and 2.45 sightings per individual on average. No individuals tagged in the first half of the nesting season were recaptured in the second half. This implies that the cohort tagged between July and November was completely replaced by a new cohort within the 123 days between the last census day in November and the first census day in March. There was only one recapture of a marked turtle between two breeding seasons. That individual was recaptured in October 1997 four years after it had received a tag while it was nesting on Enu Island in November 1993 and was subsequently observed to successfully make three nests.

The period of time elapsed between consecutive observations of individual turtles attempting to nest ranged from 1 – 113 days (n = 623, mean 15.4 ± 0.95), which confirms that it would have been highly unlikely to encounter tagged individuals during the period after the observation gap of 123 days between the two census periods. The vast majority of turtles were seen only once or twice within the time span of 1 week (63.7% of all observations), but 10.4% were observed over a time span of more than 8 weeks. Observed re-nesting intervals (the time elapsed between two consecutive successful nesting attempts) ranged from 10 – 109 days (n = 175, mean 25.3 ± 1.2 days), and 51.4% of the females returned within 10 – 20 days (n = 94, mean 14.9 ± 0.3 days). Within this range, over 76.6% of the females re-nested within 12 – 18 days.

Population size estimates for the 1997/1998 cohort are hampered by the absence of saturation tagging or 100% observation coverage of the entire rookery. In addition, the shifting of nest site preference renders sub-sampling at a pre-determined section of the total nesting area invalid. Therefore, the best estimate is obtained from a combination of the information obtained from the track examinations, successful nesting attempts and observations of the tagged individuals. The track examinations reveal a 19.48% nesting success rate

a



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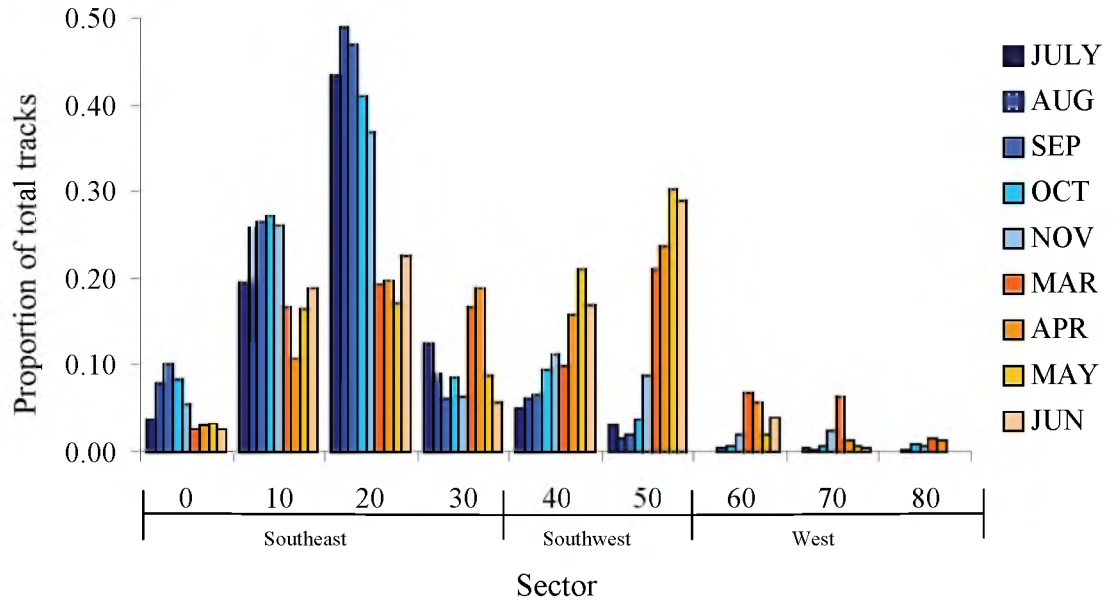


Figure 2.5. Total daily track-counts on Enu Island during the 1997/1998 breeding season (a). Data between late-November and mid-March are largely missing because of the climatological limitations to visiting the Island (see text). Spatial and temporal variation of tracks is presented in (b), with tracks in each sector (see Figure 2.1) made in the first (July to November 1997, blue) and second half of the nesting season (March to June 1998, orange). The number of tracks is expressed as a proportion of each month's total observed tracks.

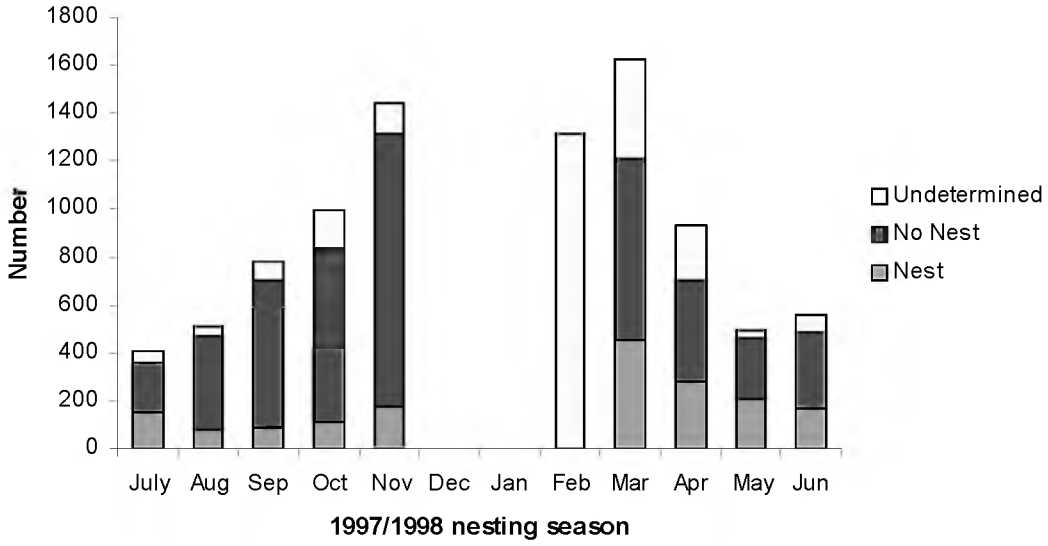
(1018 successful nests out of 5225 tracks), while observations of marked individuals reveal a slightly higher success rate of 28.40% (391 successful nests out of 1377 observed attempts). The 1.46 discrepancy between direct and indirect estimated nesting success rates then suggests that the total number of turtles in the 1997/1998 cohort would be close to 825 individuals (1.46 * 564).

The majority of all nesting attempts were unsuccessful (66%). Turtles arriving in the first half of the season produced fewer clutches (13% successful) compared to those present in the second half of the season (31% successful, Figure 2.6.a). The proportion of tracks that led to successful nesting attempts decreased from 38 to 11 % from July to November, but this proportion had increased to 28% by March and increased further to 40% towards the end of the nesting season. Unsuccessful nesting was mainly caused by failure to dig a nest in the dry sand, which collapsed into the egg chamber, or as a result of disturbance during the digging (e.g. coral rubble or wood in the sand). The warm and dry southeasterly weather conditions apparently present unfavourable nesting conditions in that time of the year, further aggravated by the hot and dry El Niño conditions of 1997 (Figure 2.7). El Niño-Southern Oscillation (ENSO) is a global coupled ocean-atmosphere phenomenon influenced by important temperature fluctuations in surface waters of the tropical Eastern Pacific Ocean. Altered trade wind circulation causes high rainfall along the west coast of South America and much drier conditions than usual in the Western Pacific (including Indonesia). The El Niño is often followed by La Niña conditions, characterised by abundant rain over South East Asia.

The onset of the rain in February, moistening the sand, abruptly changed the conditions of the substrate. Digging a nest became much easier during the second part of the nesting season, reducing the unsuccessful attempts by 29%. Turtles which failed to nest, generally returned for another attempt on the same night or within the next few nights.

There was a clear preference for nesting in sector 20 (Figures 2.5b and 2.1) in the first half of the nesting season during the southeast monsoon. During the second half of the season (March to June), most tracks were observed in sector 50 (Figure 2.5b), located along the western beach of the Island. Nesting intensity and success varied among the years (Figure 2.6b). The proportion of successful nesting attempts (combined results of observed turtles and track examination) was lowest in the 1997/1998 season, clearly reflecting the impact of the El Niño weather conditions. More clutches were deposited in the 1999/2000 season (n = 230; Figure 2.6b) than in any of the other census periods, suggesting that this cohort was the largest observed within the entire study period. Limpus and Nicholls (1988 and 2000) found a

a)



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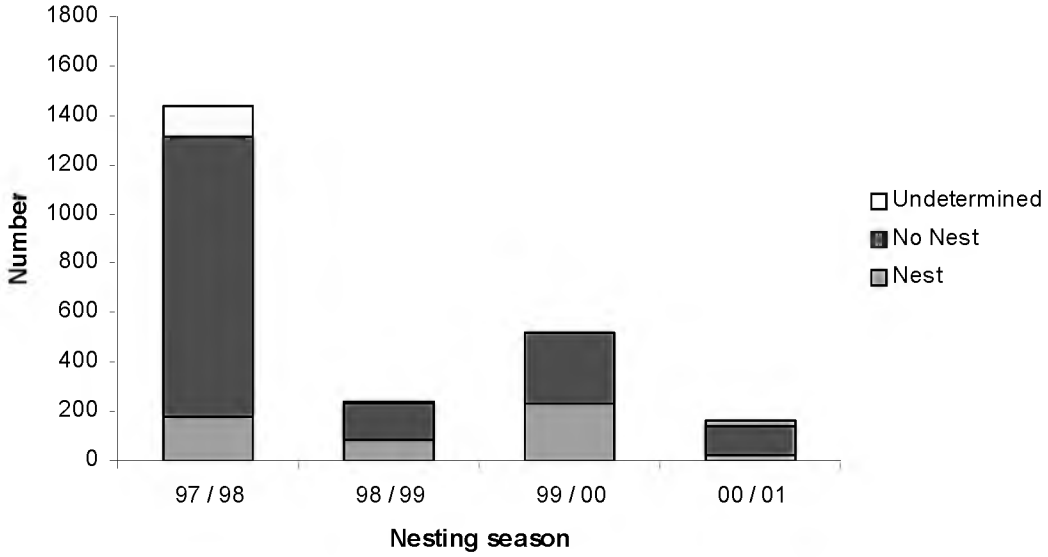

Figure 2.6. Number of tracks, expressed in successful (nest), unsuccessful (no nest), and undetermined nesting attempts on Enu Island during the 1997/1998 nesting season (a), and during the month of November across four seasons (b). Monthly numbers within and between seasons were corrected for the number of census days carried out within each month.

significant correlation between the SOI two years before the breeding season, and the number of females recorded at nesting beaches in the nGBR. Also in our area we found this correlation between the census data for Enu Island for 1993 (n = 3000; Schulz 1993), 1997 (n = 800), 1998 (n = 400), 1999 (n = 1050) and 2000 (n = 90) (see Figure 2.6b) and SOI-data from 1991 (-10.6), 1995 (-0.6), 1996 (6.3), 1997 (18.1) and 1998 (9.5; Bureau of Meteorology Australia, http://www.bom.gov.au/climate/current/soi-1993-2000.shtml), which found a correlation equation of $y = -0.0889x + 6.189$, with $r^2 = 0.6265$ and $p < 0.001$.

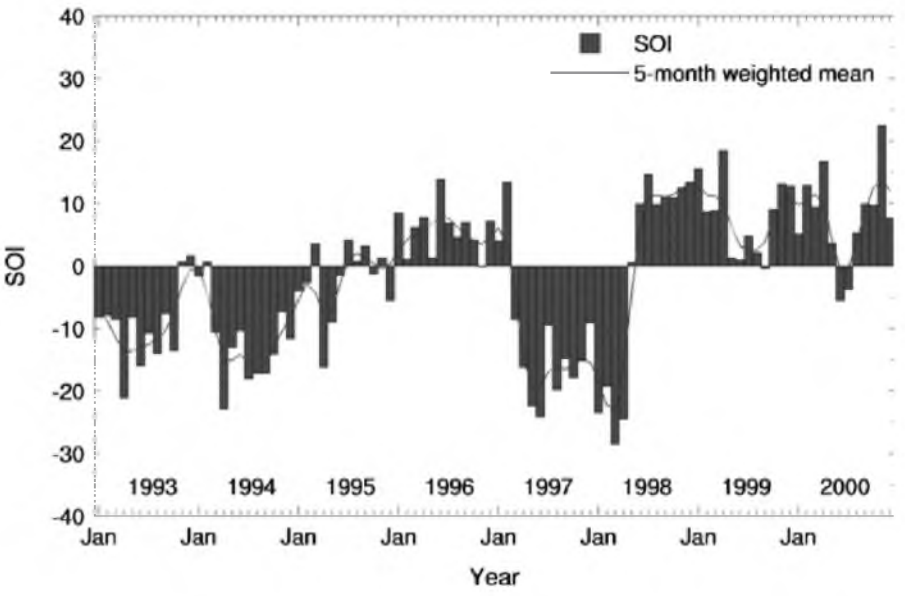

Figure 2.7. Fluctuation in Southern Oscillation Index (SOI) from January 1993 – 2000 (Bureau of Meteorology Australia, http://www.bom.gov.au/climate/current/soi-1993-2000.shtml)

The average size of all tagged adult nesting females is 103.6 cm CCL (n = 623) and ranged from 84.3 – 117.2 cm CCL (Figure 2.8). The mean clutch size was 106 ± 22.5 (n = 48) and was positively correlated with body size as $y = 1.74x - 73.89$ with $r^2 = 0.13$ ($P = 0.01$ at $\alpha = 0.05$). The mean incubation time was 58.6 ± 5.3 days, with a mean hatching success rate of 85.2 ± 12.8% (n = 91).

Of all successful nesting attempts, 72% of the nests were made in 100% sand substrate, 23% in sand with some coral rubble, 4% in coral rubble with some sand, and 1% in 100% coral rubble.

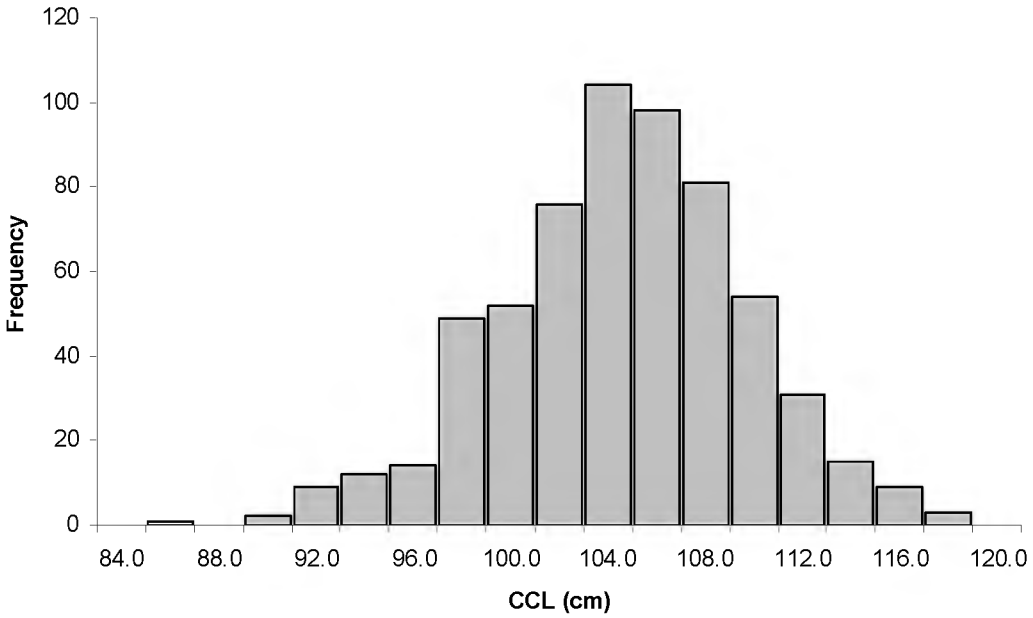

Figure 2.8. Frequency distribution of the curved carapace length (CCL) of all tagged nesting females at Enu Island between July 1997 and April 2000 (n = 623).

The hatching and emergence experiment showed that the hatching success among the four substrate-types was not significantly different in both experimental ($p = 0.228$) and natural nests ($p = 0.991$, Table 2.1). However, hatching success in the experimental nests was significantly lower that in the natural nests ($p < 0.001$), caused by increased predation pressure in the experimental set-up. Emergence success, only measured in the experimental nests, differed significantly among the different substrate types ($p = 0.004$) and was lower in coral rubble (CC) compared to sand (SS) or to coral with sand (CS), $p = 0.002$ and 0.003 respectively. Hatchling mortality was therefore also significantly higher in coral rubble than in the other substrates ($p = 0.002$). No significant differences were observed for incubation periods among substrates within the experimental nests, or compared to the donor nests.

Table 2.1. Hatching and emergence success and incubation time in various substrate types and in experimental, donor and natural nests (see Figure 2.3 b for abbreviations). Significant values for comparisons ($P < 0.05$) are indicated with asterisks

Nest location		Experiment			Donor			Natural		
	Substrate	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.
Hatching Success (%)	SS	6	72.04	10.74	9	64.14	13.72	27	90.40	2.45
	SC	6	64.04	15.79	6	87.67	7.55	12	93.36	1.31
	CS	6	38.57	10.17	2	91.21	0.66	9	93.87	1.06
	CC	6	59.13	13.32	5	79.74	7.41	6	94.89	0.80
	Overall		59.19	6.49		76.47	6.55		92.27	1.24
Hatching difference among substrates (χ^2, df = 3)			0.228			0.504			0.991	
Hatching difference among set-ups (MannW.U test)	Experiment		--			0.053			<0.001*	
	Donor		--			--			0.007*	
Emergence Success (%)	SS	6	100.00	0.00						
	SC	6	72.22	18.09						
	CS	6	97.92	2.08						
	CC	6	47.66	12.93						
Emergence difference among substrates (χ^2, df = 3)			0.004*							
Hatching difference among substrates (MannW.U test)		SC	CS	CC						
	SS	0.14	0.32	0.002*						
	SC	--	0.40	0.17						
	CS	--	--	0.003*						
Hatchling mortality (%)	SS	6	0.00	-	9	0.00	-	27	0.00	-
	SC	6	0.00	-	6	0.00	-	12	0.00	-
	CS	6	0.00	-	2	0.00	-	9	0.00	-
	CC	6	21.40	5.97	5	5.90	4.61	6	0.15	0.15
Mortality difference among substrates (χ^2, df = 3)			<0.001*			0.008*			0.73	
Mortality difference among substrates within set-ups		SC	CS	CC	SC	CS	CC			
(MannW.U test)	SS	--	--	0.002*	--	--	0.002*			
	SC	--	--	0.002*	--	--	0.029*			
	CS	--	--	0.002*	--	--	0.14			
Mortality difference among set-ups (χ^2, df = 2)	SS		0.193							
(tested for separate substrates)	SC		0.632							
	CS		0.641							
	CC		0.005*							
Mortality difference within CC among set-ups	Experiment		--			0.055			0.003*	
(MannW.U test)	Donor		--			--			0.072	
Incubation period (days)	SS	6	58.20	0.49	9	58.70	2.93			
	SC	6	58.00	1.53	6	56.75	0.87			
	CS	6	57.80	1.36	2	58.25	2.25			
	CC	6	56.00	1.36	5	56.05	0.50			
	Overall		57.73	0.55		57.57	1.02			
Incubation difference among substrates (χ^2, df = 3)			0.425			0.610				
Incubation difference among set-ups (MannW.U test)		Donor	0.767			--				

Discussion

Timing of nesting activity

A peak in the nesting season – as determined by track counts and successful nesting attempts in the 1997/1998 season – occurs from November to March (Figure. 5a and 6a). The timing of this peak coincides with nesting peaks observed for Sukamade, southeastern Java (Arinal 1997), the eastern Australian rookeries Raine Island (Limpus *et al.* 2003), Bramble Cay (Limpus *et al.* 2001) and Heron Island (Bustard 1972), as well as that observed at Barrow Island, western Australia (Pendoley 2005). However, there is considerable variability in the timing of *C. mydas* breeding throughout Australasian region. For example, at Bountiful and Wellesley Islands in the southern Gulf of Carpentaria (GoC) nesting occurs year round with a mid-year peak from July to September (Garnett *et al.* 1985). In Sarawak (western Borneo) and in the Philippine Turtle Islands a peak in nesting activity also occurs from July to September (as summarized in Chaloupka 2001). While the exact reason for this variability in timing of nesting remains uncertain, it has been hypothesized that temperature at the nesting environment (Guinea 1994), genetic variation among nesting populations (Limpus 2003), and/or climate at the feeding grounds might have an influence. At Enu Island the peaks in nesting activity, observed in November and March, coincide with the NW monsoon and the onset of rainfall. Similar climatic situations occur in the northern parts of Australia, where the build up for the wet season commences in early October. The NW monsoon brings the most severe weather conditions for Aru, which reduces the number of human visitors to the island. Selective avoidance of the nesting habitat by *C. mydas* in the months prior October may drive the timing of the nesting peak. However, high levels of anthropogenic impact in this region are a recent phenomenon and it is therefore more plausible that the timing of nesting is driven by longer term selection for more favourable condition of the nesting substrate during the NW monsoon. The favourable nesting conditions, in combination with a reduced impact from harvesting of adult animals or eggs during this period, result in an associated increased survival probability.

Nesting population size

Global rookery size data are classified into nine size classes (see Turtle Interactive Mapping System (UNEP/CMS http://www.unep-wcmc.org and the Marine Turtle Database maintained by C.J. Limpus at Queensland Parks and Wildlife Service). Following this system, the observation of 564 green turtles on Enu Island during the 1997/1998 nesting season, would rank the Aru population in the size class of 500 – 1000 nesting individuals per year. The 1997/1998 census data is considerably more conservative than the first reported population size estimate of 50 to 100 nesting individuals every night (Compost 1980). However, these

1979-estimates need to be interpreted with some caution; the report does not provide the methodology with which the "observed number of *C. mydas* nests" was determined and could be interpreted as either turtles observed to make a nest, or as a nest based on sand-disturbance, or as a track. Furthermore, the estimate was based on a three-day census period. Our study clearly shows the large fluctuations in the daily number of turtles that ascend the beach (Figure 2.5a) associated with the tidal pattern (Figure 2.2). A minimum of a two-week census covering the full high and low tide extremes is the minimum requirement in order to make a reliable estimate of the nesting population size. A more reliable estimate by Schulz (1996) suggests that a population of 2000 to 3000 turtles attempted to nest on Enu Island in 1993. *C. mydas* nesting populations are characterized by highly variable census counts in successive breeding seasons (Limpus *et al.* 2003). This annual variation is evident in tagging-data from *C. mydas* nesting along the east coast of Australia (Limpus 1995, Limpus *et al.* 2003), track counts at Barrow Island in western Australia (Pendoley 2005) and in clutch-frequency data from Sukamade, southeast Java (Arinal 1997). Despite the inherent fluctuations in the size of annual breeding cohorts, a trend in the nesting census data suggests that the Enu population has decreased substantially over the period of 1979 to 2000.

The Enu population appears to fluctuate in synchrony with fluctuations observed in eastern and western Australian populations. For these Australian populations the 1997/1998 season was an average year, 1998/1999 and 2000/2001 were extremely low, while 1999/2000 was a peak year. Schulz' estimates also fit in with this synchrony, because 1993/1994 appeared to be a peak year on Enu Island, similar as observed on Raine Island (Limpus *et al.* 2003). Numbers of *C. mydas* nesting on the eastern Australian rookeries were found to be a function of the El Niño Southern Oscillation climatic events (Limpus & Nicholls 2000; Limpus & Nicholls 1988). The El Niño Southern Oscillation Index (SOI) is calculated from the monthly or seasonal fluctuations in the air pressure difference between Tahiti and Darwin. These negative values are usually accompanied by sustained warming of the central and eastern tropical Pacific Ocean, a decrease in the strength of the Pacific Trade Winds, and a reduction in rainfall. Sustained negative values of the SOI often indicate El Niño episodes (Figure 2.7). Limpus and Nicholls (2000) found a significant negative correlation between the mean May-October SOI (MOSOI) 1.5 years before the breeding season and annual *C. mydas* census numbers. Drier and warmer conditions typical of an El Niño event reduce the productivity at foraging areas, resulting in a reduced proportion of adult females capable of breeding in the following year. The time delay between an El Niño event and the onset of breeding is thus determined by the duration of the sequence of physiological processes that culminate in egg production (Limpus & Nicholls 2000). The significant correlation between the natural logarithm of the estimated number of green turtles nesting in Aru and the MOSIO of 2 years

before the nesting event showed that the size of the annual nesting population could be predicted. The sustained negative SOI values in 2006 (http://www.bom.gov.au/climate/) suggest that a relatively large population of turtles can be expected to nest in the 2008/2009 season.

Nesting success

The average size of the nesting females on Enu Island is 103.6 cm CCL (Figure 2.8). The smallest individual measured 84.3 cm (tag number X5291) was first observed when it attempted to access the beach from the reef at low tide. It was subsequently observed on another three occasions and successfully deposited two clutches of eggs (October 8th and 21st). The individual is small compared to the rest of the individuals nesting on Enu and also compared to other nesting populations in the region. For example, the smallest female observed to nest on Raine Island was 86.0 cm CCL (mean = 105.97 cm, range 86.0 – 130.1 cm, n = 20947. Limpus *et al.* (2003). The smallest gravid female *C. mydas* at a feeding ground in eastern Australia (Shoalwater Bay) was 87.8 cm (Limpus *et al.* 2005). Conversely, sub-adult *C. mydas*, not ready to reproduce, of larger size classes have been observed in eastern Australia (e.g. Heron Reef, Limpus & Reed 1985). No damage to the carapace was observed, and the animal was measured on several occasions, precluding possibilities of false measurements. The overall smaller size of individuals nesting on Enu Island suggests that this population reaches maturity at a smaller size than for example the populations on the east coast of Australia. The single observation of one extremely small reproducing individual supports this hypothesis.

While the majority of *C. mydas* nested in sandy substrate, at least 1% of all successful nesting attempts are attributed to a clutch deposited in 100% coral rubble. However, the coarse texture of the substrate reduces the visibility of tracks and signs of digging. The percentage of nests deposited in coral rubble could therefore be larger than what has been observed. The coral-digging individuals were generally small in size (less than 100 cm CCL), suggesting that this involves younger and possibly less experienced individuals. The experiment also showed that the coral-substrate did not influence hatching success but did impact hatchling emergence. Hatchlings emerging from this substrate were generally smaller in size and weighed less than the hatchlings emerging in the other substrates. Some had not fully stretched, and the spatial emergence pattern appeared more scattered. The weight and size of the large particles probably reduces 'social facilitation' (Carr 1984), the process of simultaneous upward digging activity of the hatchlings, leaving each of the hatchlings to dig for themselves.

Annual fecundity is typically estimated based on the number of clutches per female. The inferred 15-day re-nesting interval is the most reliable estimated in the absence of a full saturation-tagging program, which was not feasible for the Enu rookery. The renesting interval at Enu Island is slightly longer than that observed for Bramble Cay (12.4 days), however, the time-span within which ≥ 75% returns to lay a next clutch of eggs falls within the primary range of 9 – 18 days as observed for the nGBR rookery (Limpus *et al.* 2001). It is therefore possible that a turtle that remains in the vicinity of the rookery for 113 days could have deposited 8 clutches of eggs. Based on the timespan spent in the vicinity of the rookery and the inferred primary renesting interval, it was possible to estimate the number of clutches potentially made by the population of turtles attempting to nest on the Enu rookery in the 1997/1998 season. The vast majority (74%) only made one nest (mean 1.3 ± 0.03), which is less than the reported mean of 3 clutches per female (Miller 1997). 24.6% accounted for making 2 – 5 nests, while 1.4% made between 6 – 8 nests. While it is possible that some turtles laid a clutch at another beach, several visits to the other islands around Enu did not reveal large numbers of nests or even tracks. It is also possible that some turtles dropped a clutch at sea after several unsuccessful attempts at digging a nest, and the extreme weather conditions may have influenced this. There was no commercial exploitation during at least the first half of the census period, which discounts the possibility that turtles were removed from the cohort while the census took place. It, thus, remains unclear what caused this low fecundity, but it is of major concern for the survival of this population.

In summary, in the Aru population of *C. mydas*, an average of 500 to 1000 female green turtles deposit between 2 to 8 clutches of around 106 eggs each year. Hatching success (92%) is highest and hatchling mortality lowest on predominantly sandy substrates as opposed to coarser substrates. A female at a size of at least 84.3 cm CCL can be expected to have reached sexual maturity. The peak of the nesting season occurs from November to April and nest site preference shifts around this island following the change in monsoons. Impact of exploitation can now be assessed based on this baseline information and conservation action more systematically planned and implemented.

3.

The genetic structure of Australasian green turtles (*Chelonia mydas*): exploring the geographic scale of genetic exchange

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Abstract

Ecological and genetic studies of marine turtles generally support the hypothesis of natal homing, but leave open the question of the geographic scale of genetic exchange and the capacity of turtles to shift breeding sites. Here we combine analyses of mtDNA variation and recapture data to assess the geographic scale of individual breeding populations and the distribution of such populations through Australasia. We conducted multi-scale assessments of mtDNA variation among 714 samples from 27 green turtle rookeries and of adult female dispersal among nesting sites in eastern Australia. Many of these rookeries are on shelves that were flooded by rising sea levels less than 10,000 years (c. 450 generations) ago. Analyses of sequence variation among in the mtDNA control region revealed 25 haplotypes, and their frequency distributions indicated 17 genetically distinct breeding stocks (Management Units) consisting either of individual rookeries or groups of rookeries and in general separated by more than 500 kilometres. The population structure inferred from mtDNA was consistent with the scale of movements observed in long-term mark-recapture studies of east Australian rookeries. Phylogenetic analysis of the haplotypes revealed five clades with significant partitioning of sequence diversity ($\Phi = 68.4$) between Pacific Ocean and SE Asian/Indian Ocean rookeries. Isolation by distance was indicated for rookeries separated by up to 2000 km but explained only 12% of the genetic structure. The emerging general picture is one of dynamic population structure influenced by the capacity of females to relocate among proximal breeding sites, though this may be conditional on large population sizes as existed historically across this region.

Introduction

Green turtles (*Chelonia mydas*) are long-lived, herbivorous reptiles with a circumtropical distribution. Understanding the complex life history of this species has been a major challenge to biologists because of the spatial and temporal scales involved. Knowledge of population dynamics is largely obtained from long-term mark-recapture studies of females tagged while nesting on the beaches. These studies show that breeding female turtles display high fidelity to the same nesting beaches (Hendrickson 1958; Carr & Ogren 1960 and Carr 1984) hypothesized that mature nesting female turtles were selecting their natal beach to deposit eggs. Studies in the southern Great Barrier Reef (sGBR) demonstrate that green turtles also display fidelity to resident feeding grounds throughout their adult lives (Limpus *et al.* 1992) with females reaching sexual maturity at about 40 years (Limpus & Chaloupka 1997). Recaptures of tagged turtles on distant feeding grounds provided evidence that at least a proportion of the green turtle population migrates over geographically large distances between nesting and feeding habitat (Dizon & Balazs 1982; Meylan 1982; Limpus *et al.* 1992), and this is corroborated by satellite tracking data (e.g. Papi *et al.* 1995; Craig *et al.* 2004). In spite of the nesting site fidelity, female green turtles have been observed to deposit clutches on different nesting beaches within and among breeding seasons (e.g. Limpus *et al.* 2003), but the geographic scale over which females switch nesting beaches remains unclear.

Analysis of mtDNA structure in Atlantic green turtle populations supported the natal homing hypothesis, as geographically distant rookeries were found to have heterogeneous mtDNA haplotype frequencies (Bowen & Avise 1996). Similarly Norman *et al.* (1994) found significant geographic structuring of mtDNA variation among eight of nine widespread rookeries studied in the Australasian region and FitzSimmons *et al.* 1997aa) inferred that male green turtles, like females, are philopatric to natal regions. The observed heterogeneity of mtDNA haplotypes among regional rookeries indicates the presence of multiple distinct genetic stocks (Bowen *et al.* 1992; Norman *et al.* 1994; Encalada *et al.* 1996) or "Management Units" (MUs; Moritz 1994a). An understanding of female movements as provided by mtDNA is of particular relevance to defining such MUs because colonisation by females is crucial to maintaining viability of meta-populations following disturbance (Avise 1995).

One limitation common to all of the above mtDNA studies is that they rarely compare closely spaced (< 500 km) rookeries, so that the extent of exchange on a local scale has not been determined. A second general limitation of such studies is that, despite the emphasis placed on mtDNA differentiation (e.g. Moritz 1994b; Avise 1995), there is considerable uncertainty

in estimating migration rates from mtDNA (Whitlock & McCauley 1999; Ballard & Whitlock 2004). In this context, it is highly desirable to combine mtDNA evidence with mark-recapture data to test for congruence. Other than broad-scale comparisons (e.g. Meylan *et al.* 1990), this has not yet been done for marine turtles. Thus, despite decades of tagging and genetic studies, we do not yet know the geographic scale of an individual breeding population; is it a single beach, immediately adjacent beaches, or a whole archipelago? Resolution of this question is essential to understanding how green turtles respond to changes in the availability of nesting sites over time, for example when nesting beaches are lost due to increasing sea levels as a result of climate change.

For green turtles, comprehensive distribution and movement patterns are being elucidated by tag-return data, but information from these studies is skewed by tagging and survey effort and typically applies only to adult females. Although the sGBR green turtle population appears to be increasing (Chaloupka & Limpus 2001), populations throughout much of the Australasian region are declining (Sloan *et al.* 1994;Limpus 1997). This has been attributed to various causes including the harvest of eggs and turtles for food, by-catch in fisheries activities, loss of nesting habitat and diseases such as fibropapillomas (Limpus & Parmenter 1985; Kennett *et al.* 1998; Broderick 1998; Dethmers 2000). In Pangumbahan (Java) and Berau (east Kalimantan) the dramatic decrease in egg production as a result of extended egg concession practices is well documented (Wicaksono 1992; Sloan *et al.* 1994; Limpus 1997). More dramatic is the situation on Bali where a complete loss of all green turtle rookeries has resulted from local overharvesting of eggs and turtles (Schulz 1984). The precipitous decline of some green turtle populations over the past few centuries (Limpus 1995) has increased the need to detect geographic boundaries and identify demographically independent populations for management.

The present study examines the mtDNA variation across 27 green turtle rookeries in Australasia (West Pacific Ocean, East Indian Ocean, and the SE Asian seas), representing all major breeding sites in the region. To test the assumption that variation in mtDNA haplotype frequencies predicts low rates of exchange, we compare genetic inferences against available recapture data, especially for the intensively studied east Australian populations. The Australasian region is especially relevant to examining potential for relocation of rookeries following sea level change as many of the sites studied are on shallow platforms that were only available to marine turtles from < 10,000 years (ca. 450 generations) ago (Figure 3.1; Torgersen *et al.* 1985; Chappell & Shackleton 1986). Sea level changes associated with glacial cycles have repeatedly exposed and flooded vast areas on the Sunda and Sahul shelves approximately every 100,000 years (Torgerson *et al.* 1985; Chappel and Shackleton 1986). As

a result, nesting habitat was created or removed. This would have resulted in repeated and large shifts of populations as gravid female turtles were forced to find new suitable nesting habitat. From patterns of sequence variation in mtDNA and available recapture data we infer population boundaries among rookeries and thereby identify MUs for Australasian green turtles to facilitate national and international management and as baseline data for subsequent analyses of foraging grounds and harvests. We also examine large-scale patterns of sequence diversity across the Indo-Pacific divide in the context of historical shifts in the location of nesting habitats.

Materials and Methods

Sampling was designed to cover all of the known major and historically important rookeries (n = 27) throughout SE Asia, Australia, the Western Pacific and Eastern Indian Oceans (Table 3.1 and Figure 3.1). For several regions (southern and northern Great Barrier Reef (sGBR and nGBR), Gulf of Carpentaria (GoC), Sulu Sea and South China Sea; Table 3.1) we were able to sample turtles from multiple adjacent rookeries. This provided a range in geographic distances among sampled rookeries from 14 to 7799 km and a total sample size of 714 individuals. Sample sizes varied extensively (9 - 60 per rookery) due to nesting population size and logistic constraints. DNA was extracted from skin tissue or blood from nesting females or from hatchlings (including samples used in Norman *et al.* 1994 and FitzSimmons *et al.* 1997bb), ensuring that progeny from a given female were only sampled once. Skin biopsies were stored in a NaCl saturated solution of 20% DMSO and blood cells were either frozen or suspended in a long-term storage buffer (100 mM Tris, 100 mM NaCl, 10 mM EDTA.2Na, 0.5% SDS).

Over the past three decades many thousands of green turtles along the east coast of Australia received self-piercing, self-locking tags initially made of monel and later of titanium, as part of a long-term mark-recapture study (Limpus 1992). Tagging data from the 1998-1999 nesting season, were analysed to estimate movement patterns of nesting females among rookeries. Distances between rookeries in the nGBR range from 5 to 460 kilometres and in

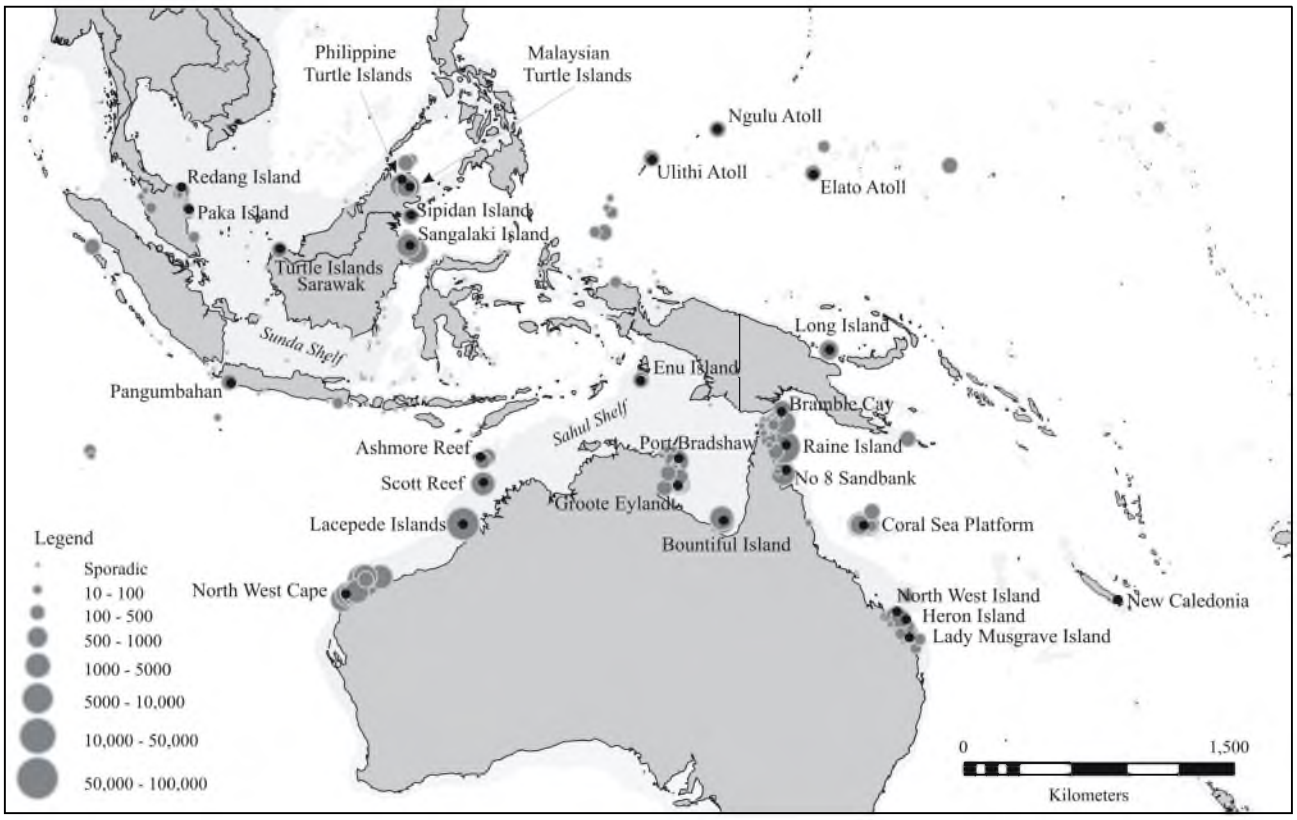


Figure 3.1. Rookeries sampled (black circles) for this study and distribution of green turtle rookeries throughout the Australasian region (grey circles). Estimated annual number of females at each of the rookeries is proportional to the size of the circles as indicated in the legend. Rookery size data have been derived from the Marine Turtle Interactive Mapping System (UNEP/CMS http://www.unep-wcmc.org) and the Marine Turtle Database maintained by C.J. Limpus at Queensland Parks and Wildlife Service. Shaded area denotes approximate landmass contours at sea levels 120 m below current levels.

Table 3.1. Frequencies of green turtle (*Chelonia mydas*) mtDNA haplotypes among 27 Australasian rookeries. Haplotype nomenclature follows Norman *et al.* (1994) and Broderick (unpublished data) and is based on Mse1 restriction digest patterns. Numbers I - V refer to clades in Figure 2.

					I										II			III				IV	V				
Sea/Ocean	**Country**	**Region**	**Location**	**Year**	**C1**	**C2**	**C3**	**C4**	**C5**	**C7**	**C8**	**C9**	**C14**	**D2**	**B1**	**B3**	**B4**	**C12**	**C13**	**J1**	**J2**	**B5**	**A1**	**A2**	**A3**	**A4**	**A6**
SW Pacific Ocean	Australia	NGBR	Bramble Cay	'90											22	2		2						1			
	Australia	NGBR	Raine Isl.& no8 Sandbank	'89				1							20					2	1			1				
	Australia	Coral Sea	Coral Sea Platform	'92	1		1									9								30			
	Australia	SGBR	Heron Island	'89												2								42			
	Australia	SGBR	Lady Musgrave Island	'90												3								21			
	Australia	SGBR	North West Island	'90												3								31			
	New Caled.		New Caledonia	'93			1								4	2			1					2			
NW Pacific Ocean	Micronesia	Elato Atoll	Elato Atoll	'92																			4		9		
	Micronesia	Ngulu Atoll	Ngulu Atoll	'92																			2	1	11	1	
	Micronesia	Yap	Ulithi Atoll	'92																			1	1	6	1	
	PNG	PNG	Long Island	'89			1			1															16		
S China Sea	Malaysia	Penins.Malaysia	Paka Island	'93			14															1					
	Malaysia	Penins.Malaysia	Redang Island	'93			8	1					1									2					
	Malaysia	Sarawak	Sarawak Turtle Islands	'91			2	18														2					
Sulu Sea	Malaysia	Sulu Sea	Mal. Turtle Islands	'93			5							23													
	Philippines	Sulu Sea	Phil. Turtle Islands	'93			8		1					30													
Celebes Sea	Indonesia	Berau	Sangalaki Island	'00			7		9				5	7													1
	Malaysia	SE Sabah	Sipidan Island	'93			18		3				1	8													
Arafura Sea	Indonesia	Aru	Enu Island	'00									27												1		
	Australia	GOC	Bountiful Island	'93	21		17				11																
	Australia	GOC	Groote Eylandt	'99	14		7				1		1														
	Australia	GOC	Port Bradshaw	'98	30		21				8														1		
Timor Sea	Australia	Ashmore Reef	Ashmore Reef	'96	3		7																	1	9		
	Australia	Scott Reef	Sandy Island	'91	5		11					2												1			
E Indian Ocean	Indonesia	W Java	Pangumbahan	'91			17		6																		
	Australia	NW Shelf	North West Cape	'89	11	1	1					1	1														
	Australia	NW Shelf	Lacepedes	'87	25		2					1					1							1			
Total					110	1	148	20	19	1	20	4	36	68	46	21	1	2	1	2	1	5	7	133	53	2	1

the sGBR from 12 to 442 kilometres. There is only rare nesting throughout the 620 kilometre stretch of the Great Barrier Reef that separates the southern most rookery in the nGBR (No. 7 Sandbank, unsampled) and the northern most rookery in the sGBR (Cockermouth and Bushy Islands, unsampled) (Figure 3.1).

DNA was extracted from small amounts of tissue (typically 0.1 g) or blood (~ 10 ml) and prepared for PCR as described in Norman *et al.* (1994). A 384 bp segment of the mtDNA control region was amplified using TCR5 and TCR6GC primers (modified after Norman *et al.* 1994, with the latter primer containing a 41bp GC clamp). Typically, 1-2 ml of template was used in 25 ml PCR reactions using standardized conditions of denaturing at 94° C for 10s, annealing at 56° C for 30s and extension at 72° C for 40s for 32 cycles.

To process the large number of samples, we developed a rapid yet sensitive screening protocol using denaturing gradient gel electrophoresis (DGGE; Myers *et al.* 1987) to detect DNA polymorphisms. Polymorphism was detected after 14 hours of electrophoresis at 80V and 58° C through a 1 x TAE 6.5% polyacrylamide gel whose denaturant gradient ranged from 30 to 45% (32% formamide and 5.6 M urea). Sensitivity was increased by hybridizing candidate DNA variants with known sequence variants of similar mobilities to differentiate homo- versus hetero-duplexes. A mixture of equal quantities (2.5 ml) of candidate and known sequence variants was denatured at 90° C for 5min., followed by ten minutes gradual cooling from 70° C to 50° C. For one group of sequence variants with a similar melting behaviour we used outgroup heteroduplexing (Campbell *et al.* 1995). The gels were silver stained, and archived. The screening strategy we employed was to (i) score samples relative to the homoduplex mobility of all known mtDNA haplotypes, (ii) confirm their identity using heteroduplex analysis, and (iii) sequence representatives from each genotype/locality combination for final verification and to test sensitivity.

Sequencing was facilitated by the use of M13 forward (gagcggataacaatttcacacagg) and reverse (agggttttcccagtcacgacgtt) universal primers that annealed to complementary tails that were added to the TCR5 and TCR6 (without the GC-clamp) primers. Dye terminator cycle sequencing was done in 10 ml reactions with 1.6 mM of the M13 primer and 30 cycles of denaturing at 96° C for 20s, annealing at 50° C for 20s and extension at 60° C for 4min. on an ABI 3730 automated sequencer. Each specimen was sequenced in both directions.

Sequences were aligned using Clustal X (Thompson *et al.* 1997) and population genetic parameters estimated in Arlequin 2.000 (Schneider *et al.* 2000). Estimates of nucleotide (p) and haplotype (h) diversity, pairwise FST tests (10,000 replicates; Slatkin 1991), exact tests of

population differentiation (100,000 replicates; Raymond & Rousset 1995) and AMOVA (10,000 replicates; Excoffier *et al.* 1992) were used to quantify genetic diversity. In the AMOVA, rookeries were grouped by their identified Management Units (stocks) and by ocean basin. Both sequence-based (ΦST) and conventional FST distance measures were used to calculate within and among population diversity.

The program Modeltest 3.7 (Posada & Crandall 1998) was used to choose among models of sequence evolution that best fit our data. The dataset considered in this paper is a subset of the global diversity of sequence variants described in *C. mydas* (Broderick unpublished data). We therefore used our data in combination with a global dataset of *C. mydas* sequence variants (selected from GenBank, Broderick unpublished data) to determine the most likely of 56 substitution models. Heuristic maximum-likelihood and a maximum parsimony analysis using a TBR branch-swapping algorithm with 200 and 1000 bootstrap replicates respectively were used to test the robustness of the inferred phylogeny. Phylogenetic analysis were performed using the program Paup* 4.10b (Swofford 2001). Parsimony haplotype networks were estimated in Arlequin 2.000 and graphically represented with the assistance of the TCS program (Clement *et al.* 2000).

Isolation by distance (IBD), under a two dimensional stepping-stone model, was tested in GenAlEx (Peakall & Smouse 2001) using conventional regression analysis (natural log [ln] of sea distance (km) versus genetic differentiation (FST/(1 - FST); Rousset 1997) and the statistical significance of the correlation was tested using Mantel's test (5000 iterations; Mantel 1967). Patterns of spatial genetic autocorrelation were examined over increasing distance classes and significant departures from spatially random distributions were detected by permutation 1000 replicates in GenAlEx. A computational geometry approach as implemented in Barrier (Manni *et al.* 2004) was used to further explore the spatial genetic landscape and identify areas where genetic barriers between adjacent rookeries might be located. Shortest sea distance between rookeries was calculated using the great circle distance that incorporates the curvature of the earth. We refrained from use of coalescent methods to estimate population parameters (e.g. theta, growth, migration) as exploratory analyses revealed very unstable results, as expected for single locus estimates (Kuhner *et al.* 1998)

Following the simple approach of Lahanas *et al.* (1994), long-term, female effective populations sizes (Nef) were compared with current effective population sizes (Naf; estimates of annual numbers of breeding females) in each MU. Nef was estimated using the relationship Nef , where m is the mutation rate per generation. A range in mutation rates from 0.006 to 0.012 substitutions per site per million years was taken from Encalada *et al.* (1996). We

expressed Nef in number of turtles per year by maintaining a generation time of 40 years as estimated for green turtles nesting along the east coast of Australia (Limpus & Chaloupka 1997) and an average interbreeding period of 5.5 years (Limpus *et al.* 1994b). Naf was derived from the Marine Turtle Interactive Mapping System (UNEP/CMS http://www.unep-wcmc.org) and the Marine Turtle Database maintained by C.J. Limpus. We used the sum of median values of population estimates where multiple rookeries occurred within a MU.

Results

Haplotype identification using DGGE

Of the 25 distinct haplotypes identified via direct sequencing, only three sets of haplotypes could not be resolved using routine heteroduplexing. Haplotypes C1, C3, C14 were subsequently distinguished using outgroup heteroduplexing (Campbell *et al.* 1995) with the C12 haplotype, providing distinct heteroduplex band patterns. Haplotypes C4 and C8 had identical melting behaviour under all conditions and were distinguished using the restriction enzyme Sau96I. Haplotypes E1 and E2 differed by a single base-pair (bp) but were indistinguishable by heteroduplexing and were grouped together as the E1/E2 haplotype in further analyses. Sequencing of representatives of all haplotypes from all populations revealed no additional sequence variants. This confirmed that our DGGE assays provided a highly sensitive, repeatable and cost effective strategy to rapidly screen for variants in marine turtle populations. It is important to note that this work was carried out when sequencing was still very expensive for us and that the same methods were used to screen mtDNA variants in thousands of samples from feeding grounds and harvests (K. Dethmers, D. Broderick, unpublished data).

The best-fit model of sequence evolution as selected by the hierarchical likelihood ratio tests (hLRTs) was the Hasegawa–Kishino–Yano model (Hasegawa *et al.* 1985) (HKY + G + I). The second best-fit model was Tamura-Nei (Tamura & Nei 1993) (TrN), with G = 0.25 and I = 0.45. We adopted the simpler TrN model in subsequent analysis because it is supported in most population genetic data analyses software packages.

Mitochondrial DNA diversity and phylogenetic structure

Screening of polymorphism within the 384 bp mtDNA control region fragment among 714 turtles from the 27 rookeries revealed 25 distinct haplotypes (Table 3.1 and GenBank accession numbers S76889 and AY955198 - AY955221; haplotype nomenclature follows Norman *et al.* 1994 and is drawn from a global analysis of mtDNA variation in green turtles;

Broderick *et al.*, in prep.). Of 45 polymorphic sites, 41 were transitions, one site contained both a transition and a transversion and three sites were characterised by indels. A single base insertion distinguished the B5 haplotype and a single base deletion was unique to the A1 haplotype. The E1 and E2 haplotypes shared a 10 base-pair (bp) direct duplication not seen in the other haplotypes. This same duplication was reported among phylogenetically independent haplotypes from Atlantic Ocean rookeries (Lahanas *et al.* 1994). The 25 haplotypes differed by between 1 and 35 observed mutations, corresponding to estimated sequence divergences of 0.3% – 10% (mean = 5.2%). Maximum-likelihood and maximum-parsimony trees produced similar topologies with the latter tending to have higher levels of bootstrap support for each node (Figure 3.2). There were five well-supported clades on the phylogenetic tree with one to ten haplotypes each, separated by 3.3% - 8.4% mean sequence divergence (Figure 3.2). Within clades, sequence divergence was low (0 – 1.3%). The 95% confidence parsimony networks could be constructed within, but not across the five clades because of large inter-clade divergences (Figure 3.2).

Population diversity and subdivision

Despite the identification of five divergent clades, phylogeographic structure was diminished by the occurrence of several widespread haplotypes (Table 3.1, Figure 3.2). For example, the numerically dominant (and in the network, centrally located) C3 haplotype of Clade I was widely distributed across the Indian Ocean and SE Asia, and had limited occurrence in the Pacific Ocean. The rest of Clade I (C1 etc.) and Clade V (A1 etc.) were also widespread.

Clade I was predominant in the Arafura Sea rookeries and across the Sunda Shelf to the east Indian Ocean. Clade V predominated in the Pacific Ocean, but also occurred in rookeries of the western Indian Ocean. Clade II (B1/3/4) occurred mostly, but not exclusively, in Pacific Ocean rookeries, as did Clade III (C12/13-J1/2). Clade IV (B5) was only observed from the Sunda Shelf samples.

Exact tests for divergence of haplotype frequencies among all 27 rookeries revealed that 17 of the 351 pairwise comparisons were non-significant ($P > 0.05$). Of the non-significant comparisons, nine involved geographically proximal rookeries: Heron, Lady Musgrave and North-West islands of the sGBR (≤ 96 km apart, P = 0.34 - 0.68); Raine Island/No.8 Sandbank and Bramble Cay from the nGBR (274 km apart, P = 0.56); Bountiful Island, Groote Eylandt and Port Bradshaw from the Gulf of Carpentaria (≤ 567 apart km, P = 0.17 - 0.56); Paka and Redang islands from Peninsular Malaysia (133 km apart, P = 0.14); and the Malaysian and Philippine 'Turtle Islands' (14 apart km, P = 0.76). Two sets of comparisons

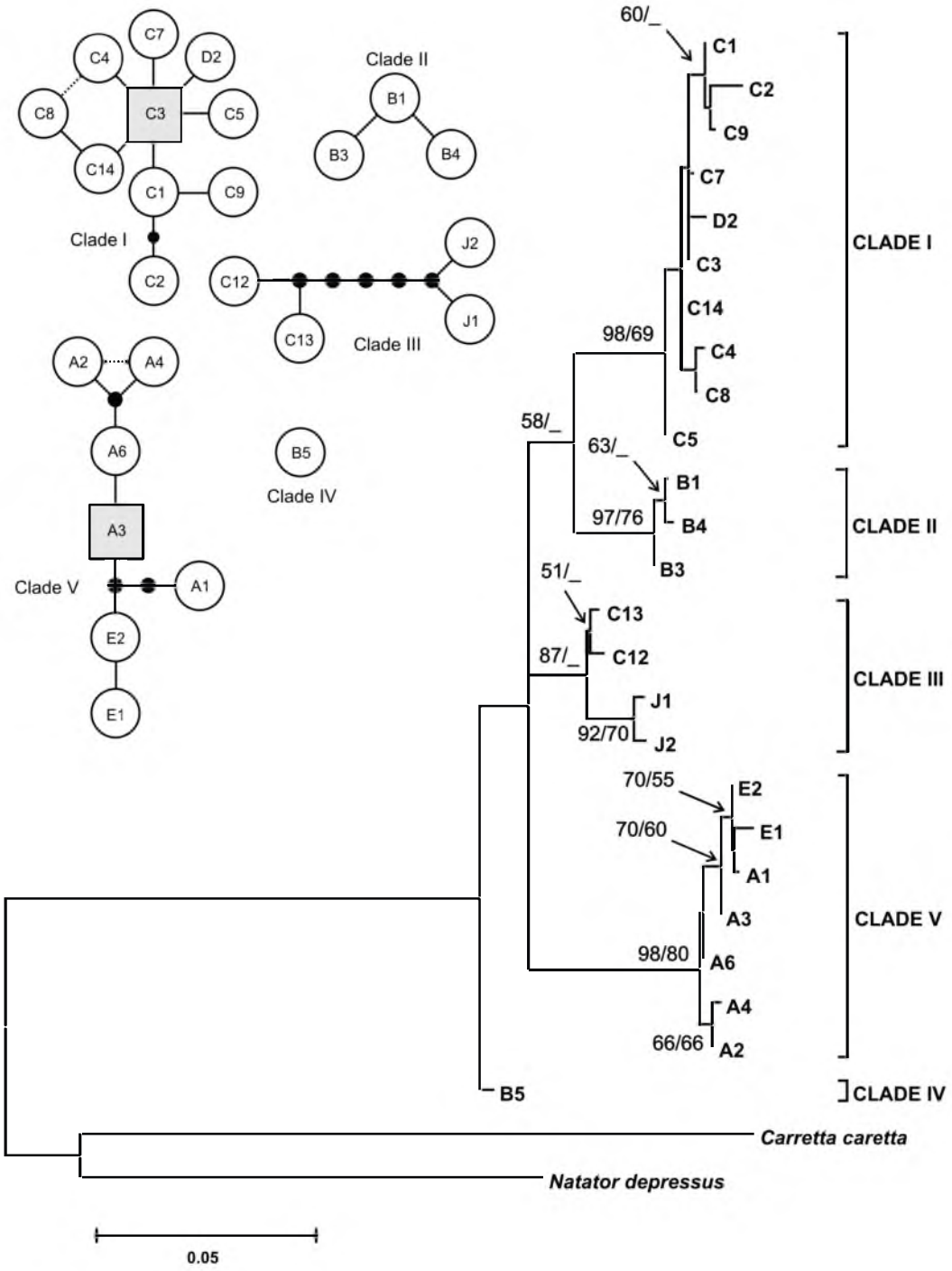


Figure 3.2. Green turtle phylogeny and the estimated 95% set of plausible parsimony networks (top left) describing the relationships among 25 mtDNA control region haplotypes. The phylogeny shows percentage bootstrap support from a maximum parsimony (before slash) and a maximum likelihood heuristic search (after slash) using TrN distance measures and 200 and 1000 bootstrap iterations respectively. Branch lengths are proportional to the percent sequence divergence indicated by the scale. Haplotypes within the haplotype network connected by solid lines are one mutational step away from each other; alternative

parsimonious connections are represented by dotted lines. Presumed ancestral haplotypes are represented by shaded squares and unsampled intermediate haplotypes are represented by showed no significant divergence in haplotype frequencies between more distantly separated rookeries. The first involved two rookeries from the North-West Shelf of Australia, the Lacepede Islands and the North-West Cape Jurabi coast (997 km apart, P = 0.79). The second involved the three Micronesian rookeries of the Elato, Ngulu and Ulithi atolls ($\leq$ 1026 km apart, P = 0.22 – 0.71). Each of the remaining four non-significant (0.18 < P < 0.75) pairs of comparisons involved a single rookery from one of the above statistically homogeneous groupings and another distant rookery. We combined geographically proximal rookeries that did not have significantly different haplotype frequencies (i.e., Peninsular Malaysia, Sulu Sea, Micronesia, North West Shelf, GoC, nGBR, and sGBR). Using these groupings, the Exact tests were repeated, now solid circles. Haplotype nomenclature follows Norman *et al.* (1994) and Broderick *et al.* (in prep) and is based on Mse1 restriction digest patterns.
among 17 population groupings. Of the 136 pairwise comparisons, all were significant at a P = 0.05 threshold after correction for multiple tests using the sequential Bonferroni method (Rice 1989). Throughout the rest of the analyses these 17 groups were considered to represent MUs (Figure 3.3).

Haplotype diversity varied widely among the 17 MUs (h = 0.07 – 0.82) and showed little consistent variation among regions (Table 3.2). Nucleotide diversity was substantially lower in the SE Asian MUs than the Indian Ocean MUs and these were both lower than the Pacific Ocean MUs (p = 0.006, 0.019 and 0.034 respectively; Table 3.2). This difference reflects the dominance of closely related haplotypes from Clade I in the SE Asian and, to some extent, the Indian Ocean populations, whereas the Pacific Ocean populations include haplotypes from multiple, distantly related clades (Table 3.1, Figure 3.2).

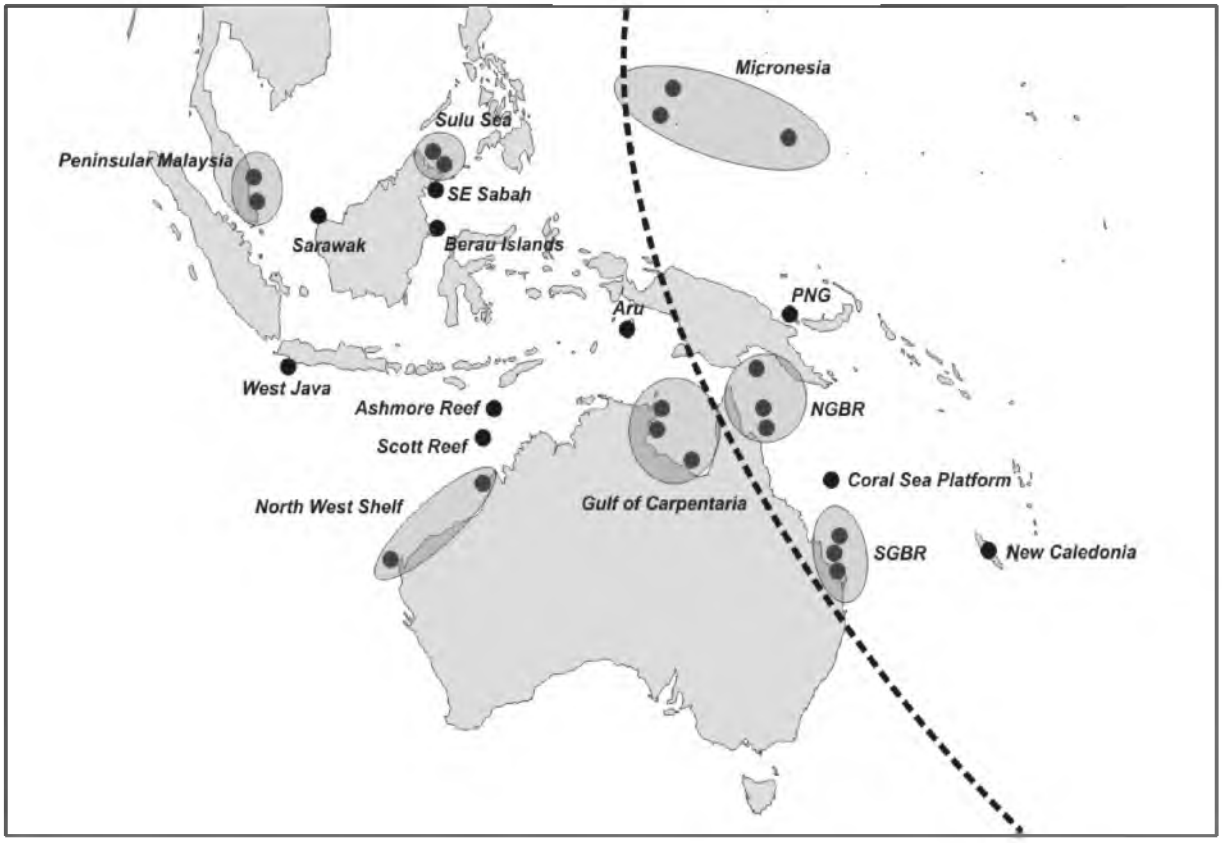


Figure 3.3. Location of 17 genetically distinct breeding stocks or Management Units as inferred from analysis of geographic structure of mtDNA variants and position of the genetic barrier (dashed line), indicating the major genetic discontinuity between the Pacific Ocean rookeries from those to the west.

Table 3.2. Estimates of haplotype ($\hat{h}$) and nucleotide (π) diversity among 17 green turtle genetic stocks and estimates of effective population sizes. N_{af} is an estimate of the current population size. N_{ef} is the range of estimated effective population sizes based on mutation rates of $\mu = 0.009$ substitutions/site/million years.

Region	Stocks (MUs)	n	?	s.e.	π	s.e.	N_{ef}	N_{af}	N_{ef}/N_{af}
Pacific	nGBR	52	0.35	0.08	0.019	0.010	4725	24300	0.19
	Coral Sea	41	0.43	0.08	0.030	0.015	7465	2800	2.67
	sGBR	102	0.15	0.04	0.010	0.006	2525	6600	0.38
	New Caledonia	10	0.82	0.10	0.042	0.023	10678	?	-
	Micronesia	49	0.68	0.06	0.004	0.003	987	1300	0.76
	PNG	18	0.22	0.12	0.017	0.009	4255	800	5.32
Pooled Pacific stocks		272	0.71	0.02	0.034	0.017			1.86
SE Asia	GOC	132	0.62	0.02	0.004	0.003	1026	6600	0.16
	Aru	28	0.07	0.07	0.006	0.004	1408	1000	1.41
	Berau Islands	29	0.78	0.03	0.008	0.005	2063	7100	0.29
	SE Sabah	30	0.58	0.08	0.002	0.002	447	300	1.49
	Sulu Sea	67	0.34	0.06	0.001	0.001	248	13900	0.02
	Sarawak	22	0.33	0.12	0.011	0.006	2842	300	9.47
	Peninsular Malaysia	27	0.33	0.11	0.012	0.007	3118	350	8.91
Pooled SE Asian stocks		335	0.80	0.01	0.006	0.004			3.11
Indian	Ashmore Reef	20	0.68	0.06	0.045	0.023	11349	600	18.92
	Scott Reef	19	0.61	0.10	0.010	0.006	2576	300	8.59
	West Java	23	0.40	0.09	0.001	0.001	273	300	0.91
	North West Shelf	45	0.36	0.09	0.007	0.397	1645	125300	0.01
Pooled Indian stocks		107	0.70	0.03	0.019	0.010			7.11
Overall Australasia		714	0.88	0.01	0.041	0.020			

Comparisons between estimates of long-term, female effective populations size (Nef) derived from nucleotide diversity values and current estimates of the total number of nesting females (Naf) were equivocal. The ratio of Nef / Naf within MUs ranged from 0.01 – 18.92 (Table 3.2). In the 16 comparisons that could be made, eight indicated that Nef > Naf, six indicated that Nef <Naf , and in two comparisons the estimate for Naf fell within the range estimated for Nef. The average ratio of Nef / Naf within MUs was 3.72 (median = 1.16) but there was a considerable range within each region. For example the Indian Ocean MUs had both the highest average ratio of Nef / Naf (7.11) and the largest range (0.01 – 18.92) while the Pacific Ocean MUs had the lowest average ratio of Nef / Naf (1.86) and the lowest range (0.19 – 5.32).

Of the 25 haplotypes we identified, 44% occurred uniquely in the Pacific Ocean, and 8% and 20% occurred uniquely in the Indian Ocean and SE Asian regions, respectively. The latter two regions were the least structured sharing 58% of haplotypes and the net sequence divergence between these regions was low (0.11%) compared to that between the Pacific and the Indian Ocean and SE Asian regions (4.03% and 5.16% respectively). Analyses of Molecular Variance among MUs and among regions (Indian Ocean, Pacific Ocean, and SE Asia) indicated strong genetic structure ($P < 0.001$; Table 3.3). Overall, the proportion of variation distributed among the 17 MUs was higher (78%) when molecular differences among haplotypes were included than when treating haplotypes as equidistant (53%), suggestive of some underlying separation of evolutionary lineages. The most striking effect was observed in a hierarchical analysis comparing regions; only 10.3% of genetic variation was partitioned among regions if considering only haplotype frequencies, versus 61.4% if also considering haplotype divergence. However, this pattern was not consistent within regions. The incorporation of haplotype divergence into the AMOVA made no difference for the Indian Ocean comparisons, and it decreased the proportion of variance distributed among SE Asian MUs. Regardless of the approach, greater genetic variation within versus among MUs was indicated for the Indian Ocean and SE Asia and the opposite was indicated for the Pacific Ocean. The Barrier analysis identified a major genetic discontinuity separating all Pacific Ocean rookeries from those to the west (Figure 3.3). This phylogeographic break is also evident in AMOVA analyses; partitioning Pacific Ocean MUs from the other 11 MUs explains the greatest amount of genetic variation (68.4%) whereas only 1.54% of sequence variation was partitioned between Indian and SE Asian MUs.

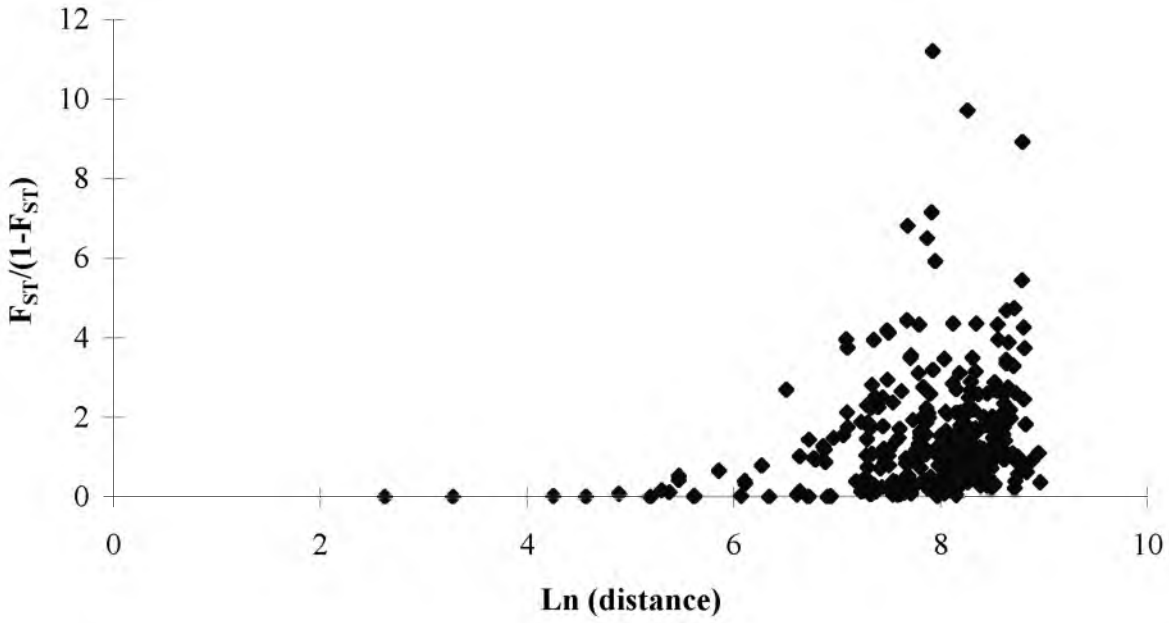
Isolation by distance and migratory behaviour

A Mantel test for positive association between distance matrices of genetic structure (FST/(1 - FST) and geographic distance was significant ($P = 0.001$) among the 27 rookeries sampled (Figure 3.4a). However, the correlation (r^2) only explained 6% of the variance in these data. Analysis of spatial genetic autocorrelation indicated that genetic similarities between populations were significant when these populations were separated by up to 2000 km ($P = 0.011$; Figure 3.5). At larger geographic distances there was no relationship between genetic and geographic distance. When correlation analyses were repeated only for rookeries separated by not more than 2000 km (using 1000 replicates), the correlation value (r^2) doubled to 12% (Mantel $P < 0.001$, Figure 3.4b).

Table 3.3. Partitioning of molecular variance (%) of green turtle MUs at multiple geographic scales. All values are significant ($P < 0.001$).

		Φ_{ST} (TrN)			Conventional F-Statistics		
	n	Among regions	Among stocks	Within stocks	Among regions	Among stocks	Within stocks
All stocks	17		77.6	22.4		52.8	47.2
Pacific stocks only	6		61.8	38.2		57.6	42.4
Indian stocks only	4		39.9	60.1		39.9	60.1
SE Asian stocks only	7		29.2	70.3		45.3	54.7
Stocks within regions		61.4	20.7	17.9	10.3	44.1	45.6

a



b

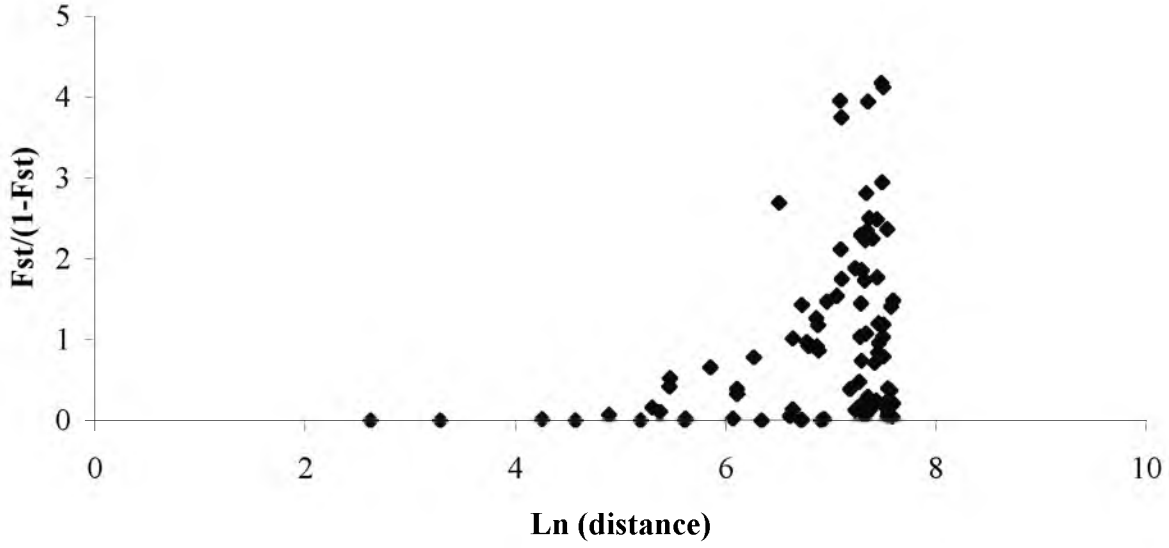


Figure 3.4. Genetic differentiation among pairs of all Australasian green turtle populations (a) and populations separated by 2000 km. or less (b), with regressions y = 0.43x - 1.97 and r^2 = 0.06; y = 0.40x – 1.72 and r^2 = 0.12 respectively. F_{ST} was linearized according to Slatkin (1993).

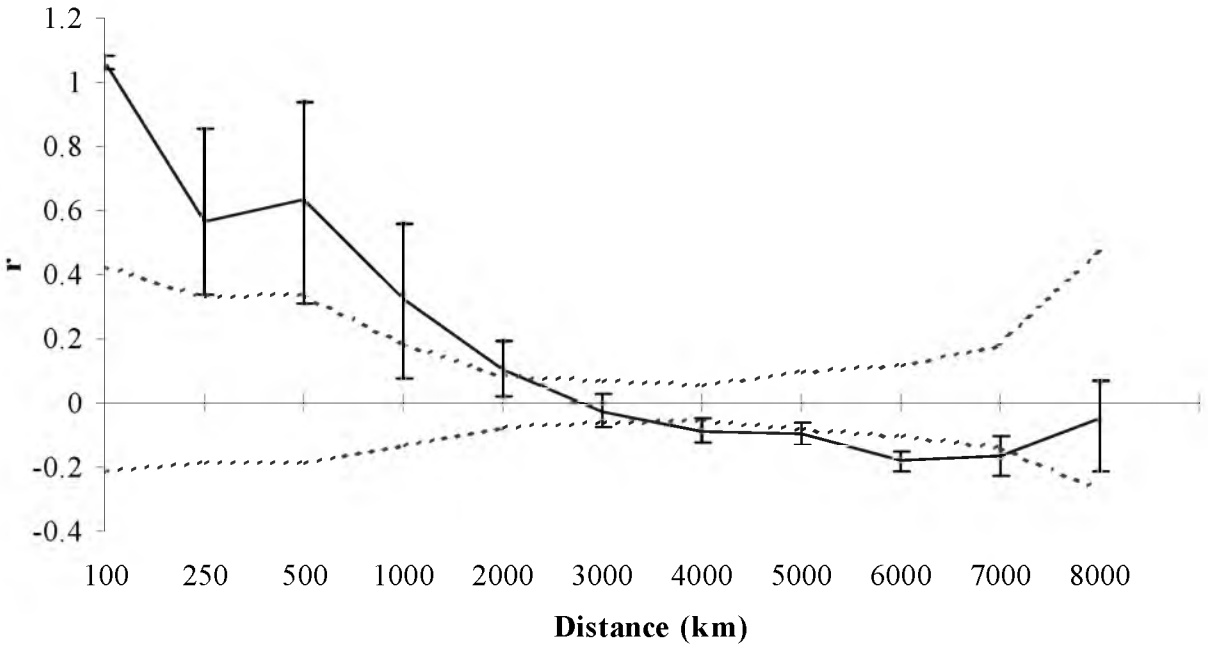


Figure 3.5. Spatial autocorrelation analysis of green turtle stocks in the Indo-Pacific. The solid line represents genetic correlation as a function of geographical distance. Dotted lines indicate the 95% confidence interval about the null hypothesis of no spatial structure and were estimated from the 1000 permutations.

Along the east coast of Australia, the summer of 1998-99 was one of the largest green turtle nesting events on record and it provided an opportunity to compare the movements by nesting females among rookeries at multiple spatial scales. A total of 8,156 tagged turtles were recorded during that season (Table 3.4), of which 2,891 were remigrants from previous seasons. Of these inter-seasonal remigrants, 171 (6%) had changed rookeries between seasons and an additional 132 females (1.6% of the total) were observed to change rookeries between successive nesting events within the season. The subsequent mtDNA analysis showed that all of the migration events occurred within the identified MUs; in particular, the geographically extensive surveys of nesting females allowed for detection of nesting migrations between sGBR and nGBR, but none were observed. The rate of rookery switching for remigrants was somewhat higher within the sGBR (151/1791 = 8.4%) than in the nGBR (23/967 = 2.4%). Distances between alternative rookeries visited interseasonally by a single turtle ranged from 17 to 266 km (mean = 42 km) in the sGBR and from 12 to 218 km (mean = 65 km) in the nGBR. Within seasonal shifts between rookeries occurred over distances of 17 to 188 km (mean = 44 km) in the sGBR and 12 to 50 km (mean = 22 km) in the nGBR.

Table 3.4. Recapture data of green turtles (*Chelonia mydas*) nesting in eastern Australia during the 1998-1999 Australian summer nesting season, collected as part of an extensive combined State and Federal tagging program. Values represent the number of turtles displaying between seasonal rookery fidelity (diagonal), between seasonal rookery switching (other) and within seasonal rookery switching (in parentheses). Saturation tagging of the total nesting population only occurred at Heron Island, Milman Island and Mon Repos. Other rookeries were sampled at mid season only.

	Rookeries where turtles were originally tagged		Rookeries examined in 1998-1999 season																			
			MM	DS	PC	RI	N8	N7	CSC	SW	CI&FI	NW	WI	HI	HO	LM	LE	WR	RB	MP	MR	FR
Northern Great Barrier Reef Rookeries	Milman Is.	MM	96		1	3																
	Douglas & Sinclair	DS	1 & 1	-																		
	Moulter Cay	PC			1	7																
	Raine Is.	RI			7+(3)	847																
	No8. Sandbank	N8				1	-															
	No7. Sandbank	N7				3		-														
	Coral Sea cays	CSC							133+(2)													
Southern Great Barrier Reef Rookeries	Swain Reefs cays	SW								3												
	Curtis and Facing Is.	CI&FI								3	1											
	Northwest Is.	NW									1	344	1+(5)	10+(9)		0+(1)						
	Wreck Is.	WI										9+(6)	382	8+(12)		2		1				1
	Heron Is.	HI										74+(42)	16+(34)	501		4+(2)						
	Hoskyn Is.	HO													0							
	Lady Musgrave Is.	LM										2	4	4+(3)	3	383	1	1				
	Lady Elliot Is.	LE														1+(4)	0					
	Wreck Rock	WR										2				1		12				1+(1)
	Rules Beach	RB																	0			
	Moore Park	MP																		1		
	Mon Repos	MR																			1	
	Fraser Is.	FR										1				0+(5)	0+(2)					15
	Total tagged turtles		271	0	144	2069	0	0	1095	27	6	805	766	1801	76	740	99	65	4	4	3	181

Discussion

Phylogeography and population structure

Green turtle populations sampled across Australasia had high levels of genetic diversity and showed evidence of a historical split between populations in the Pacific Ocean and those in the Indian Ocean and SE Asian region. However, the phylogeographic structure between the Pacific and Indian Ocean is less extreme than that between the Indo-Pacific and Atlantic Ocean (Bowen *et al.* 1992), as might be expected given the tropical marine connections between the Indian and Pacific Oceans.

Overall haplotype diversity of green turtles in Australasia was high (h = 0.88) and similar to Atlantic populations (h = 0.83, Encalada *et al.* 1996). Nucleotide diversities were elevated in Australasia (p = 0.040) compared to the Atlantic populations (p = 0.005) sampled over a similar geographic scale. However, within the Australasian region, the SE Asian rookeries had a pattern more like the Atlantic populations with high haplotype (h = 0.80) and low nucleotide diversities (p = 0.006). For the Atlantic green turtle populations, it was suggested that this pattern of variation reflects a relatively recent colonisation by small founder groups from equatorial refugia, after sea temperatures increased at the end of the last glaciation (Encalada *et al.* 1996; see also Grant & Bowen 1998;Reece *et al.* 2005). In Australasia marine turtle rookeries would have been impacted substantially by glacial cycles; sea level changes have repeatedly exposed and flooded vast areas on the Sunda and Sahul shelves approximately every 100,000 years over the passed 500,000 years (Torgerson *et al.* 1985; Chappell & Shackleton 1986). Most of the SE Asian green turtle nesting populations are in areas that were dry land at the Last Glacial Maximum 18,000 years ago, when sea levels were 120-150 m below present (Chappell & Shackleton 1986; Voris 2000) and could only have been colonized between 10,000 and 6,000 years ago as sea levels rose to their current levels. As new nesting habitat became available with rising sea levels, the most likely source of founders is nesting females from adjacent populations. As new nesting habitat became available with rising sea levels, the most likely source of founders is nesting females from adjacent populations. The case of the Gulf of Carpentaria population on the Sahul shelf is instructive about both the process of colonisation and the potential for rapid divergence because the Gulf was flooded by marine waters from the west about 10,000 years ago (Torgerson *et al.* 1985). The distribution of mtDNA haplotypes suggests colonisation of the Gulf of Carpentaria primarily from the west (now represented by the NW Shelf MU, which is known to use this region as a feeding ground, and the Timor Sea MUs) and perhaps the north (Aru MU). What is intriguing is that the GoC population predominantly nest in the austral

winter, whereas the NW Shelf, Timor Sea and Aru turtles predominantly nest in the austral summer. It appears that some point, selective pressures have caused a shift in the timing of nesting within the last 10,000 years (c. 250 generations) as the Gulf of Carpentaria was colonised.

A strong feature of the mtDNA data for Australian green turtles is the distinction between the Pacific versus Indian Ocean and SE Asian populations. The Pacific rookeries were dominated by haplotypes from clades I and IV, whereas the others had mostly haplotypes from clades II, III and V. This qualitative pattern was reinforced by the AMOVA and Barrier analyses. During the Pleistocene the intervening Torres Strait was repeatedly exposed, forming a land barrier between northeast Australia and New Guinea and it last reopened approximately 6,000 years ago (Chappell & Shackleton 1986). Other genetic breaks due to this land barrier have also been observed in a variety of marine organisms including barramundi (*Lates calcarifer*; Chenoweth *et al.* 1998), coconut crab (*Birgus latro*; Lavery *et al.* 1996) and several other invertebrates (Benzie 1999). What is surprising is that despite recurrent opening of this barrier approximately every 100,000 yrs and the proximity of the large nGBR population, the genetic divergence has not been substantially eroded. Clearly natal philopatry at a subregional scale has provided an effective buffer against complete homogenisation of these regions over long time scales.

Our estimates of historic effective size versus current census population size produced highly variable results, but with many instances of Nef / Naf > 1. This contrasts with the general pattern of effective population sizes being an order of magnitude smaller than census sizes (Frankham 1995b), which might suggest major declines in current population size due to human impacts. Such estimates have been used previously to infer that populations are not in equilibrium due to historical fluctuations or recent reductions in population size (e.g. Lahanas *et al.* 1994). However, there are several caveats. First, the imprecision around point estimates for both Nef and Naf is likely to be large because the former is based on a single gene and the latter is based on heterogeneous survey data. Second, admixture of divergent stocks during Holocene range expansions will inflate estimates of Nef and even past admixture events can have a pronounced effect, as the time taken to return to equilibrium is proportional to Ne generations. Ideally, we would evaluate the demographic history of these populations and estimate current Nef using coalescent simulations (e.g. Kuhner *et al.* 1998; Excoffier 2004; Hamilton *et al.* 2005). However, given the small number of generations (< 250), evidently low rate of substitution in mtDNA of marine turtles (Avise *et al.* 1992), and the noise associated with parameter estimation from a single locus, we defer such analyses until a survey of microsatellite loci (N. FitzSimmons, in progress) is completed.

The geographic scale of contemporary dispersal

The combination of multi-scale and intensive analyses of recaptures from physical tagging and genetic diversity has allowed for strong inference about the geographic scale of contemporary exchange among rookeries within and among regions. In eastern Australia the tag return data from the 1998-1999 nesting season showed an 8.3% dispersal among rookeries in the sGBR between nesting seasons for distances of around 250 kilometres but no movements were observed between the nGBR and sGBR, separated by more than 1250 km. These single-season observations reflect those of long-term mark-recapture data from the major nesting sites in eastern Australia (Limpus *et al.* 1994b, 2001, 2003). Crucially, these annual surveys include multiple nearby rookeries within regions (sGBR, up to 100km; nGBR, up to 300km apart) as well as between major breeding aggregations separated by larger distances (nGBR-sGBR, 1250 km). Data from a 28-year tagging program on Raine Island and adjacent nesting sites in the nGBR showed that of 3,662 females observed nesting in across multiple years, 99% did so at the same rookery at which they were initially tagged (Limpus *et al.* 2003). Only one percent had shifted to other rookeries, mostly to nearby rookeries; Moulter Cay (n = 16; 14 km), No. 8 Sandbank (n = 10; 156 km) and No. 7 Sandbank (n = 7; 166 km). On one occasion a Raine Island turtle was recorded nesting on Bramble Cay (274 km). Similarly, 1.2% of turtles tagged while nesting on beaches within a 300 km radius from Raine Island were observed nesting on Raine Island in subsequent nesting seasons. Of particular interest, the mark-recapture surveys of the Raine Island rookery provided the only evidence that nesting movements among distant east Australian breeding sites can occur, albeit rarely. These records involved one female originally tagged while nesting on the Coral Sea Platform (850 km distant) and another originally tagged while nesting in the sGBR (at 1,700 km); both later nested on Raine Island. These two events represent just 0.05% of the remigrants recorded. Conversely, no migrants from either the nGBR or the Coral Sea MUs have been observed nesting in the sGBR over the more than 30 years of intensive surveys. These cases also provide evidence that natal homing is not obligatory for green turtles, but rather that they do have the capacity to colonise new and distant habitat. The only other region where long-term mark-recapture data is available is at the Sulu Sea rookeries of the Philippines and Malaysian Turtle Islands. Here turtles shifted between rookeries separated by less than 100 km (De Silva 1986; Trono 1993) within one nesting season.

Our genetic data are concordant with the mark-recapture data in demonstrating that gene flow among green turtle populations is likely to occur among proximate rookeries located within 500 km (e.g., within the nGBR, sGBR, GoC, Sulu Sea and Peninsular Malaysia MUs), but only rarely among more distant ones. Given this behaviour we expected that *C. mydas* would

show a pattern of isolation by distance, wherein gene flow is effective at shorter geographic distances, but genetic drift prevails over longer distances (Hutchison & Templeton 1999). Indeed significant genetic correlation was apparent at smaller spatial scales (up to 2000 km) but, compared to other species with similar dispersal capacities, the overall association between genetic divergence and geographic separation was weak. Much stronger signatures were found in Indo-Pacific tasselfish (*Polynemus sheridani*), where 45% of the genetic differentiation ($P < 0.001$) was explained by geographic distances less than 3000 km (Chenoweth & Hughes 2003). Similarly, more than 50% of genetic variation in Pacific coral reef fish (*Acanthurus triostegus*) and 62% in the northwest Atlantic cod (*Gadus morhua*) were explained by distance up to 6,200 km and 7,000 km respectively (Pogson *et al.* 2001; Planes & Fauvelot 2002). Pacific island populations of the coconut crab (*Birgus latro*) showed strong isolation by distance (60% of variation explained over 8000 km) and these were clearly different from the Indian Ocean populations (Lavery *et al.* 1996). The underlying assumption in tests for isolation by distance is that the populations under study are at migration-drift equilibrium (Wright 1943; Slatkin 1993) and, given the recent colonisations of the Sunda and Sahul shelves, we expect that the green turtle populations examined here violate this assumption. The repeated sea level changes during the late Pleistocene and associated exposure and flooding of beaches would have driven turtles to relocate and find new suitable nesting habitat, resulting in repeated and massive shifting of turtle populations across regions that at times had been isolated for thousands of years. Given the slow generation time in green turtles of 40 years (Limpus & Chaloupka 1997) and the inferred mutation rates of 0.006 – 0.012 (Encalada *et al.* 1996), the time elapsed since population colonisation (6000 years) has not been sufficient for the populations to reach an equilibrium, which partially explains the weak association between geographic and genetic distance. Again, it would be more appropriate to use multi-locus data and analytical methods that estimate contemporary or post-expansion migration rates (e.g. Pritchard & Donnelly 2001; Wilson & Rannala 2003; Hamilton *et al.* 2005).

Despite the limitations of the data for estimating population parameters, our analyses yield a useful qualitative result; the spatial scale of exchange observed in the intensive recapture study is consistent with that inferred from patterns of heterogeneity of mtDNA haplotype frequencies. Congruence between recapture results and mtDNA variation has been observed previously, but only at a broad spatial scale (Meylan *et al.* 1990). The multi-scale recapture and mtDNA evidence from eastern Australia offers some validation to the widespread practice of delimiting demographically independent "Management Units" of marine turtles on the basis of distinctive mtDNA profiles (Moritz 1994b; Avise 1995; Bowen 1995).

Implications for management

Many green turtle rookeries in Australasia have undergone precipitous declines over the last few decades, but others appear more stable (e.g. Limpus 1997; Chaloupka & Limpus 2001). Understanding the geographic scale at which rookeries are demographically connected versus independent is central to diagnosis, management and monitoring of these populations (Moritz 1994b; Bowen & Avise 1996). Our analyses of Australasian green turtles have identified 17 distinct MUs. All are present in single country jurisdictions, except for the Sulu Sea MU, which crosses the Malaysia/Philippines border. Where demographic units of nesting populations encompass multiple nations, they not only serve to focus management plans geographically but also to emphasize that conservation policies need to be regulated towards a common goal. The combination of genetic homogeneity and tag-return data across the Malaysian and Philippine Turtle Islands indicates regular interchange of females and thereby supports their joint management (Palma 1997). There is no such evidence for joint Management Units between Australia and neighbouring nations (Indonesia, New Caledonia and PNG), given that all nesting beaches for each MU are contained within a single country. Whereas the tag-return data from eastern Australia show that individual turtles move between rookeries as far as 250 km apart, evidence of genetic exchange between several rookeries within 500 km suggests that movements at this scale are not uncommon, though rarely documented. As such a 500 km range typically provides a more accurate picture of the scale at which female movements occur and provides a guideline for conservation planning processes in this region. Additionally, turtles from each of these MUs cover a much greater geographic area during development and migrations between nesting and foraging locations; for example, Limpus *et al.* (2003) reports various instances where female turtles nesting in the nGBR were recaptured in foraging locations in the sGBR at > 1500 km distance or vice versa. Similarly, turtles were captured on feeding grounds in Aru, Indonesia that were originally tagged while nesting on Raine Island in the nGBR (KD pers. obs.), or the Lacepede Islands in Western Australia (J.P. Schulz pers. comm.) and turtles nesting on sGBR beaches were recaptured at feeding grounds in PNG, Solomon Islands and New Caledonia (Limpus 1992). As these migrations often cross international borders the importance of joined international management cannot be over-emphasized. The larger countries (e.g. Malaysia, Indonesia and Australia) are typically supporting multiple MUs within their boundaries and their identification is crucial to management by local communities, state and national agencies. For example, harvesting practices at one rookery can unknowingly have an impact on multiple populations at surrounding rookeries. Delineation of management areas for each management unit relies on a combination of tag returns, satellite tracking and genetic analysis of foraging and harvested populations all of which are currently being evaluated for this region.

Two features of green turtle populations in the Indo-Pacific are i) that groups of adjacent rookeries that are isolated from other rookeries by more than a few hundred kilometres can be expected to support a genetically distinct Management Unit, and ii) where a chain of adjacent rookeries extends over a large geographical area, the entire assemblage can be expected to represent a single Management Unit. Extrapolating from the findings above we can make predictions about the genetic affinities of some genetically unsampled but regionally significant rookeries. Rookeries from the northwest coast of West Papua and the coastal areas from Thailand through to Vietnam and China are likely to form two new Management Units based on their size and distance from to other Management Units. Rookeries off the West Kalimantan coast in the South China Sea are likely to be included within the Sarawak Management Unit and would benefit from a multinational management approach similar to that covering the Sulu Sea Management Unit.

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4.

Migration of green turtles from Australasian feeding grounds inferred from genetic analyses

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Marine and Freshwater Research - Submitted

Abstract

Coastal seagrass habitats in tropical and subtropical regions support aggregations of resident green turtles (*Chelonia mydas*) from several genetically distinct source populations. Migration of individuals to their respective dispersed breeding sites provides a complex pattern of migratory connectivity among nesting and feeding habitats of the species. An understanding of this pattern is important in regions where the persistence of populations is under threat from anthropogenic impacts. This study uses mitochondrial DNA and mixed stock analyses to assess the connectivity among seven feeding grounds (n = 463, F_{ST} = 0.077) located along a 5000-kilometer transect across the north Australian coast and adjacent areas and 17 genetically distinct breeding populations from the Indo-Pacific region. Most sampled feeding grounds had proportionally large mean contributions from breeding populations of northwestern Australia, but relatively few from breeding populations of the Great Barrier Reef (GBR) to the east. While most feeding grounds appear to support multiple breeding populations, the south-western Gulf of Carpentaria was dominated by a single local genetic stock (93 ± 2.6%). Similarly, the Indonesia feeding population at Aru was dominated by the local breeding population (49 ± 8.8%), but with only a modest contribution from Australian rookeries. Geographic distance between breeding and feeding habitat negatively influenced whether or not a breeding population was present at a feeding ground (w_i = 0.654). However, neither distance nor size of a breeding population were good predictors of the extent of their contribution to the feeding ground. The differential proportional contributions demonstrate the impact of anthropogenic mortality at feeding grounds should be assessed on a case-by-case basis, but nearby nesting populations are prone to be affected.

Introduction

Long distance migration is a characteristic trait of most large marine species (e.g., whales, white sharks, and turtles). It is driven by ecological and biogeographic processes, such as the spatial and temporal distribution of resources and habitat, seasonal variation in temperature and currents, reproductive needs and differential survival across regions. The geographic extent and direction of oceanic migration within a species can vary among populations and among individuals within a population (Alerstam *et al.* 2003). The green turtle (*Chelonia mydas*) is a classic example of a migratory species. Migrations during early life history phases can involve dispersal within an entire ocean gyre and adult breeding migrations between feeding and nesting habitat may encompass thousands of kilometres (e.g. Limpus *et al.* 1992). Some populations also have a developmental migration phase, in which immature turtles leave one feeding ground to migrate to another where they mature and remain as adults (Whiting & Guinea 1998; Bjorndal *et al.* 2000). Despite a large number of studies, gaps remain in understanding the mechanisms behind selection and recruitment of individual turtles to a feeding ground. Knowledge of the connectivity between turtles in nesting and feeding habitats is required to allow quantification of the impact of threats (e.g., the geographic extent of anthropogenic mortality) with more precision, thereby enhancing the successful management of green turtles.

The green turtle is a large, long-lived, herbivorous reptile that grazes on seagrass and selected marine macroalgae in shallow tropical and temperate waters throughout the world (Bjorndal 1997). Several studies have found that aggregations of turtles at a feeding ground are derived from several genetically distinct breeding populations (Limpus *et al.* 1994, 2005; Lahanas *et al.* 1998; Bass & Witzell 2000; Luke *et al.* 2004). Each such foraging population can be referred to as a 'mixed stock'. In addition, studies of adult females have shown that individuals faithfully migrate between their breeding areas and resident feeding areas (Limpus *et al.* 1992; Balazs 1994; Godley *et al.* 2002; Troeng *et al.* 2005). Knowledge concerning the contributions of breeding populations to feeding grounds in Australia and the region comes from tagging studies (Limpus & Reed 1985a; Limpus *et al.* 1992, 1994, 2003, 2005; Prince 1997) and satellite telemetry of post-nesting females (Spring 1994; Spring & Pike 1998; Kennett *et al.* 2004). These studies have confirmed the overlap of different breeding populations at feeding grounds in Australia and showed a large variation in the extent of dispersal of turtles from breeding grounds to feeding grounds (e.g., from < 8 km to >2000 km, Limpus *et al.* 1992). However, interpretation of tag recoveries to determine the contribution of the respective breeding populations to any one feeding ground is difficult when there is

uneven tagging effort at the breeding grounds and uneven capture effort at the feeding areas. In addition, such an imbalance in mark-recapture efforts complicates investigation of factors that influence the relative contributions. For example contribution from populations nesting in close proximity to the feeding ground are generally expected to be higher than those from distant populations, and larger populations are expected to contribute more than smaller populations do.

In Australasia, most green turtle populations experience anthropogenic mortality on the feeding grounds to various degrees. The harvest of green turtles in northern Australia, Papua New Guinea and eastern Indonesia is believed to represent the greatest threat to the green turtle stocks in this region (Limpus & Chatto 2004). Commercial green turtle harvests take large numbers of turtles at feeding grounds to be sold on regional markets in PNG (Limpus & Parmenter 1985), Indonesia (Dethmers 2000) and the Philippines (R. Cruz, pers. Comm). There are also non-commercial harvests of green turtles through much of Australasia, including harvest by local indigenous communities in Australia (Kowarsky 1982; Johannes & MacFarlane 1991), PNG, Indonesia (Suarez & Starbird 1996) and Melanesia (e.g. the Solomon Islands, Broderick 1998). In principle, an assessment of the genetic composition of turtle feeding populations can provide insights into the identity of genetically distinct populations impacted and the extent to which each is affected by such harvests. Understanding the composition of feeding grounds in this region is made possible because of recent surveys of mitochondrial DNA (mtDNA) variation that included 27 green turtle rookeries within the Australasian region, and identified 17 genetically distinct breeding populations, including seven in Australia (Dethmers *et al.* 2006).

In this study we use mtDNA variation and mixed stock analysis to examine the relative contributions of green turtle breeding populations to assemblages at multiple feeding grounds across the north Australian coast and adjacent areas. We examine patterns of regional migratory connectivity of green turtles between breeding areas and feeding grounds and test whether population size or proximity to feeding grounds can be used as predictors for population representation at feeding grounds in Australasia. From these results, the geographic extent of possible threatening processes impacting on green turtle populations can be more readily estimated for the region. This type of study, together with existing knowledge about regional green turtle stock distributions and migrations, is important for understanding the potential consequences of threatening processes and management practices for turtles at both breeding and feeding habitats.

Methods

Sampling

Seven green turtle feeding grounds were selected across the northern region of Australia and south-eastern Indonesia to represent an east-west sampling transect (Figure 4.1). Three of these feeding grounds are adjacent to significant nesting beaches of known genetic composition; Ashmore Reef (AR), Aru Islands (AI) and the Sir Edward Pellew Islands (SEP). Feeding grounds at Cobourg Peninsula (CP), Field Island (FI) and Cocos (Keeling) Islands (CK) are adjacent to beaches that only have sporadic nesting and are thus considered as remote feeding grounds. Fog Bay (FB) is remote from green turtle nesting beaches and supports only immature turtles, thus it is considered a developmental feeding ground (Whiting & Guinea 1998). Turtles were captured using drift nets, barramundi gill nets, by hand while walking on reef flats and by the rodeo method (Limpus & Reed 1985b). Once captured, turtles were tagged with a unique numbered titanium tag to prevent double sampling. Skin biopsies were taken from the dorsal surface of the shoulder and stored in a NaCl saturated solution of 20% dimethylsulfoxide. Curved carapace length (CCL) was measured along the midline from the junction of the skin and carapace at the neck to the posterior margin of the carapace (Limpus 1985). We used a CCL of 84.3 cm as a cut-off point to distinguish between resident and potential non-resident turtles, the latter being individuals that have migrated there to breed. This cut-off point is based on the smallest turtle observed (Dethmers *et al.* in prep. a) to be nesting on Aru. It should be noted that this is a conservative size limit for this study; sub-adult green turtles of larger size classes have been observed in eastern Australia (e.g. Heron Reef, Limpus & Reed 1985b) and the average size observed in female green turtles preparing to breed for the first time at a feeding ground in eastern Australia (Shoalwater Bay) is 97.9 cm (minimum 87.8 cm; Limpus *et al.* 2005).

Molecular methods

Methods for DNA extraction and genotyping followed those used in a regional study of breeding populations (Dethmers *et al.* 2006). DNA was extracted from small amounts of tissue using the "salting out" procedure (Miller *et al.* 1988) and resuspended and stored in a 1 x TE and 5% chelex solution. A 384 bp segment of the mtDNA control region was amplified using TCR5 (5' ttgtacatctacttatttaccac) and TCR6 (5' caagtaaaactaccgtatgcc) primers (modified after Norman *et al.* 1994, with the latter primer containing a 41bp GC clamp). Typically, 1-2 µl of template was used in 25 µl PCR reactions using standardized conditions of denaturing at 94° C for 10s, annealing at one-cycle, 1° C touchdown temperatures from 59-56° C for 30s and extension at 72° C for 40s for 32 cycles. Haplotypes were identified using

denaturing gradient gel electrophoresis (DGGE; Myers *et al.* 1987) as described in Dethmers *et al.* 2006). The sensitivity of the DGGE screening protocol was increased through in-group and out-group heteroduplex analysis and targeted sequencing, thus minimizing the possibility of cryptic haplotypes. Selected samples were sequenced in both directions on a CEQ2000 capillary sequencer for haplotype confirmation with the use of M13-tailed TCR5 and TCR6 (without the GC-clamp) primers (Dethmers *et al.* 2006).

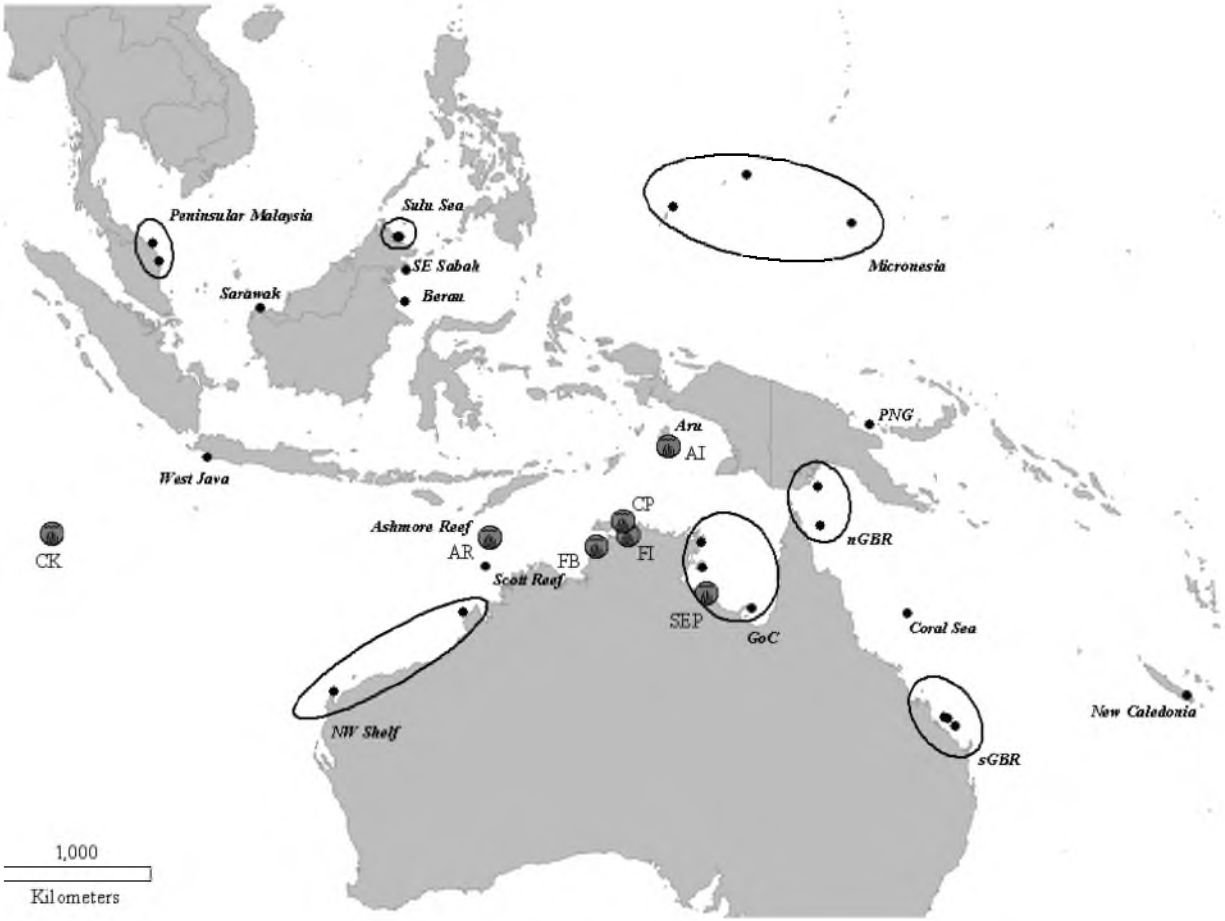

Figure 4.1. Location of samples from feeding grounds (larger filled-in circles) of green turtle (*Chelonia mydas*) aggregations in Australasia, relative to genetically distinct breeding populations. Feeding grounds are abbreviated as follows; CK = Cocos Keeling, AR = Ashmore Reef, FB = Fog Bay, FI = Field Island, CP = Cobourg Peninsula, AI = Aru Islands, SEP = Sir Edward Pellew Islands. Several of the contributing stocks are abbreviated as PNG = Papua New Guinea, nGBR = northern Great Barrier Reef, sGBR = southern Great Barrier Reef, GoC = Gulf of Carpentaria, NW Shelf = Northwest Shelf.

Statistical methods

Complimentary reverse sequences were checked against forward sequences in Sequencher 4.1.4 (Gene Codes, Inc.) and final sequences were aligned using Clustal X (Thompson *et al.* 1997). These sequences were compared with those found among nesting populations in the Australasian region and if unique, compared against haplotypes provided in Genbank and at the Archie Carr Centre for Sea Turtle Research (http://accstr.ufl.edu/cmmtdna.html). Estimates of nucleotide (π) and haplotype ($\hat{h}$) diversity, exact tests of population differentiation (100,000 replicates; Raymond & Rousset 1995), pairwise F_{ST} tests, and AMOVA (10,000 replicates; Excoffier *et al.* 1992) were performed in Arlequin 3.01 (Schneider *et al.* 2000) and used to examine genetic structure across the feeding grounds. For estimates of sequence divergence the Tamura & Nei 1993) model of nucleotide substitution was used.

Proportional contributions by each of the stocks to each of the feeding grounds were determined using a maximum likelihood approach as incorporated in the software package SPAM (version 3.7b: Alaska Department of Fish and Game 2003). As potential contributors, we used the 17 genetically distinct breeding populations or groups of populations (hereafter referred to as stocks) in Australasia with distribution and genotypic frequencies described in Dethmers *et al.* (2006). While sampling of these stocks was designed to cover all of the known major and historically important rookeries (n = 27) throughout SE Asia, Australia, the Western Pacific and Eastern Indian Oceans (see Dethmers *et al.* 2006) it is possible that some genetically unsampled but regionally significant rookeries exist. Therefore, the baseline dataset is potentially not complete and additional unstudied stocks might be represented in the mixtures.

Estimates of proportional contributions were derived through two approaches as provided within the package; the standard Conditional Maximum Likelihood (CML) approach and a computational Bayesian approach as developed by Pella & Masuda 2001). The CML approach maximizes a likelihood function of the haplotypes in the mixed stock at the feeding ground, assuming that the contributing stock compositions are known without error (Millar 1987). Contrary to CML estimates, the pseudo-Bayesian model does not assume that if a source population has a sample frequency of zero for a haplotype, it is an impossible source for an individual with that haplotype. In addition, the pseudo-Bayesian model uses prior information to more accurately distinguish between source populations. It does this by using the genetic similarities among stocks to estimate relative haplotype frequencies within the separate stocks. In doing so, the observed baseline of relative haplotype frequencies of individual stocks is shrunk to establish a regional mean to help reduce estimation error (Pella

& Masuda 2001). The sampled haplotype frequencies in the mixed stocks are then used to improve the estimates of relative haplotype frequencies in the contributing stocks. This is important when analysing a series of mixed stocks using the same baseline populations (see also Bolker *et al.* 2007). The posterior mean of haplotype relative frequencies is computed as a weighted average of the observed and prior mean relative frequencies. Confidence intervals for estimated contributions in each of the mixed stocks were kept at 90%. New haplotypes, not previously detected at the contributing stocks, were removed from the analyses. While the pseudo-Bayesian method is better than CML at accommodating observations of previously unidentified (novel) haplotypes in mixed stocks (Bolker *et al.* 2003), error due to potential contributions from as-yet unsampled regional breeding populations is not overcome.

We used the output from the mixed stock analysis to test for the hypothesized influence of geographic distance and population size on the distribution of stocks across the feeding grounds. In multiple stepwise regression tests, with percentage contribution (transformed to $\sin^{-1}\sqrt{0.01 * p}$, where p is contribution in percent) as the response variable and distance ($D_{stock\text{-}FG}$) and population size (N_{stock}) as the predicting factors, the assumption that the errors are normally distributed was not met. Generalised linear models (GLMs) provide an alternative approach in which the regression is not carried out on the response variable, y, but on a linearised version of the link function applied to y (Crawley 2002). The statistical evidence for correlations between contribution and $D_{stock\text{-}FG}$ and N_{stock} were evaluated by an evidence ratio approach using Akaike weights in Program *R* (version 2.6.0, *R Development Core Team 2005*). Binomial GLM models (equivalent to ANOVA and ANCOVA) with logit-link functions were used to determine the statistical relationship between contribution (breeding population present or absent) and (i) distance ($D_{stock\text{-}FG} \approx$ 0-<500, 500-<2000, ≥2000 km), (ii) population size ($N_{stock} \approx$ 0-<500, 500-<5000, 5000- <10000, ≥10000 individuals), as well as (iii) size class of individuals in the feeding ground sample (N_{CCL}: all < 84.3, most < 84.3, most ≥ 84.3 cm). For each of the stocks that were estimated to contribute at least 10% to one of multiple feeding grounds we estimated the correlation coefficients between percentage contribution and $D_{stock\text{-}FG}$. Shortest sea distances between nesting beaches of the contributing stocks and the feeding grounds were calculated using the great circle distance equation that incorporates the curvature of the earth and by estimating the shortest distance to re-routed migratory pathways around major landmasses. Population sizes were derived from the marine turtle database maintained by CJL and previously published in Dethmers *et al.* (2006).

To determine if individuals with CCL < 84.3 cm (Ind_{small}) and those with CCL ≥ 84.3 cm (Ind_{large}) at a single feeding ground were recruiting from different stocks, pairwise Exact tests

were repeated at three of the feeding grounds (CP, AI and SEP). These were selected because sample sizes in both size classes were sufficiently large (CP: $Ind_{large} = 57$, $Ind_{small} = 34$; AI: $Ind_{large} = 20$, $Ind_{small} = 20$; SEP: $Ind_{large} = 55$, $Ind_{small} = 47$) to allow for statistical inferences.

Results

The analyses across all feeding grounds revealed 30 distinct haplotypes (Table 4.1). Of these, 14 haplotypes were previously identified among the Australasian nesting populations (Dethmers *et al.* 2006) and they represented > 95% of sampled individuals. The origin of the remaining 16 newly detected (novel) haplotypes (Genbank accession numbers EF156419 – EF156434), comprising 22 individuals and 4.75% of all observations, is not known. These new haplotypes were most prevalent (up to 15%) at feeding grounds in the Northern Territory, and varied by 1-2 base pairs from the most similar haplotypes previous observed. All fell within the 5 clades identified in Dethmers *et al.* (2006). Comparison of haplotype frequencies at feeding grounds and regional stocks (Table 4.1) reveal that feeding grounds other than Aru (AI) are dominated by the C1 and C3 haplotypes, which are widely distributed across stocks from northern Australia, the Sunda Shelf and Indian Ocean, but are rare in Pacific Ocean stocks. Conversely, the haplotypes that dominate the east Australian rookeries (southern GBR, northern GBR; haplotypes A2, B1, B3) are rare in the sampled feeding grounds. Likewise, the C4, C5 and D2 variants that (along with C3) characterise the central Sunda Shelf (Peninsula Malaysia and Borneo) stocks are at low to moderate frequencies only. Overall haplotype diversity was 0.75 (Table 4.2) and was relatively uniform across all feeding grounds; with lower values observed for SEP ($h = 0.64$), AR ($h = 0.61$) and particularly, CK ($h = 0.45$). By comparison, overall haplotype diversity among the stocks was 0.88, with a wide variation ranging from h = 0.07 – 0.82 (Dethmers *et al.* 2006). Nucleotide diversity among the feeding grounds was quite variable, ranging from 0.001 (CK) to 0.037 (AI) and an overall diversity of 0.013, which is considerably lower than the overall nucleotide diversity found among the stocks ($\pi = 0.041$).

Results of the AMOVA analysis indicated significant partitioning of genetic variance among the feeding aggregations ($F_{ST} = 0.090$, $P < 0.001$), though the majority of the variation (91%) was explained by within population variation. Exact tests for population differentiation based on haplotype frequencies (Table 3) indicated that four feeding aggregations, CP, AI, SEP and CK had unique haplotype frequencies, whereas, the AR, FB and FI feeding grounds were statistically homogeneous after sequential Bonferroni correction of alpha values. Based on this homogeneity and the geographic proximity of FB and FI (230 km distance), we have

pooled these feeding aggregations in subsequent mixed stock analysis. Analyses of adult (including residents and potential migrants) versus non-adult (resident) turtles at CP, AI and SEP, did not reveal any significant shifts in the genetic compositions; exact tests for sample differentiation based on haplotype frequencies were not significant within each of the feeding grounds ($P = 0.92$, 0.84 and 0.83 respectively).

Results of the mixed stock analyses indicated no differences in the maximum likelihood estimates as derived under the CML versus the pseudo-Bayesian models for six of the seven feeding grounds. The only case where the estimates derived with the CML approach were substantially different from those derived through the pseudo-Bayesian approach was at the CK feeding ground. Although the identification of representing stocks at CK remained unchanged, the mixed stock estimates changed: the Peninsular Malaysia stock changed from 16% to 35%, Scott Reef stock from 75% to 28% and GoC stock from 0.5% to 14% in CML and pseudo-Bayesian approaches (Table 4.4) respectively. The CK feeding ground was dominated by the widespread and thus largely uninformative C1 and C3 haplotypes (Table 4.1), which, in combination with CK's relatively small sample size, presumably contributed to the uncertainty. In addition, the East Indian Ocean contains many as-yet unsampled rookeries, which further erodes our confidence in the estimation of contributing stocks. For these reasons, we exclude the CK estimates in further analyses and discussion.

Table 4.1. Frequencies of green turtle (*Chelonia mydas*) mtDNA haplotypes at 17 Australasian Management Units (adapted from Dethmers *et al.* 2006) and at seven foraging grounds across the North Australian coastline and SE Indonesia. N_f is the estimated total number of breeding females (Dethmers *et al.* 2006)

| | | Familiar Haplotypes | | | | | | | | | | | | | | | | | | | | | | | Novel Haplotypes | | | | | | | | | | | | | | | | | | |

Table 4.2. Genetic diversity within the foraging grounds

Region	Foraging ground	N	$\hat{H}$	π
Indian Ocean	Cocos Keeling	36	0.452 ± 0.070	0.001 ± 0.001
Timor Sea	Ashmore Reef	65	0.614 ± 0.054	0.012 ± 0.007
	Fog Bay	67	0.771 ± 0.040	0.016 ± 0.008
Arafura Sea	Cobourg Peninsula	91	0.785 ± 0.029	0.027 ± 0.014
	Field Island	62	0.747 ± 0.051	0.017 ± 0.009
	Aru Islands	40	0.722 ± 0.059	0.037 ± 0.019
Gulf of Carpentaria	SEP Islands	102	0.643 ± 0.035	0.008 ± 0.005
Combined Feeding Grounds		463	0.749 ± 0.015	0.013 ± 0.007

Table 4.3. P-values of pairwise comparisons among feeding grounds based on exact tests of population differentiation derived from haplotype frequencies. Significant values ($P < 0.05$) are indicated with asterisks.

	CK	AR	FB	FI	CP	AI	SEP
Cocos Keeling	–						
Ashmore Reef	0.0009*	–					
Fog Bay	0.0001*	0.0817	–				
Field Island	0.0008*	0.8127	0.0878	–			
Cobourg Peninsula	0.0029*	0.0431*	0.0012*	0.0218*	–		
Aru Islands	0.0000*	0.0000*	0.0000*	0.0000*	0.0000*	–	
SEP Islands	0.0001*	0.0014*	0.0000*	0.0012*	0.0000*	0.0000*	–

The green turtle aggregations at each of the feeding grounds were derived from multiple breeding stocks (Table 4.4) linked to nesting beaches located from 55 km to over 2500 km distant (AI – PNG). The origin of the stocks and the range of possible proportional contributions varied among the sites. Mixed stock estimates at three of the feeding grounds (AI, SEP and AR) revealed a dominance of a single stock with a mean contribution of 50% or more. For AI and SEP this involved the geographically most proximate breeding stocks; Aru (41 - 58%) and GoC (90 - 93%) respectively, both within a distance of 200 km. However, at the Ashmore Reef feeding ground 48 to 70% of the contributions could be assigned to the NW Shelf stock, located 500 km from this feeding ground. Interestingly, the Ashmore Reef stock (at less than 50 km distance) was not represented at AR at all. In contrast, 9 - 24% of turtles at the Cobourg Peninsula feeding grounds was estimated to have originated from the

Ashmore Reef stock, at 950 km distance. The SEP feeding ground had the lowest diversity, with apparently 90 - 96% of its population nesting within the Gulf of Carpentaria. FB + FI and CP supported the highest diversity of stocks, with five or more represented at each of the feeding grounds. These feeding grounds also had the highest proportion of novel haplotypes (13 and 5).

Some of the stocks were detected across a number of feeding grounds. For example, Northwest Shelf and Scott Reef stocks contributed substantially to multiple feeding grounds across northern Australia (except for SEP). Interestingly, these stocks represent the largest and the smallest stocks in the region, with an estimated total effective population size of N = 690,000 and N = 1,500 respectively (Table 4.4). Likewise, the GoC and nGBR stocks contributed to multiple feeding grounds, though, the latter was generally weakly represented.

The most parsimonious model to explain the contribution of stocks to feeding grounds revealed that presence or absence of a population mainly was determined by the factor distance ($D_{stock\text{-}FG}$; $w_i = 0.654$). There was less support for a model based on distance as well as population size (contribution = distance + population size; $\Delta_i = 1.99$; $w_i = 0.242$). There was little support for the global model (contribution = distance + population size + size class: $\Delta_i = 3.68$; $w_i = 0.104$) and no support for the null model (contribution = population size and contribution = size class: $w_i < 0.001$). Correlation values of the relationship between percentage contribution and $D_{stock\text{-}FG}$ were determined for nGBR, PNG, GoC, Scott Reef and NW Shelf ($r^2 = 0.25$, 0.03, 0.45, 0.72 and 0.61 respectively).

Table 4.4. Estimated proportional stock contributions to five individual feeding grounds, and to the combined Fog Bay and Field Island feeding grounds. Contributions are expresses as the range of likely proportional contributions (and the mean) within a 90% confidence interval. Estimates are based on the Pella and Masuda model (2001). For definitions of abbreviations, see Figure 4.1.

Contributing Stock	Feeding Ground											
		CK		AR		FB + FI		CP		AI		SEP	
	N	36		65		129		91		40		102	
NGBR	52	0		0 - 3	(1.3)	0 - 2	(1.3)	7 - 13	(10.1)	5 - 15	(9.8)	0 - 27	(4.2)
Coral Sea Platform	41	0		0		0		0		0		0 - 3	(1.2)
SGBR	102	0		0		0		0		0		0	
New Caledonia	10	0		0		0		0		0		0	
Micronesia	49	0		0		0		0 - 5	(2.3)	0		0	
Papua New Guinea	18	0		2 - 8	(4.8)	4 - 8	(5.7)	0		15 - 30	(22.1)	0	
Gulf of Carpentaria	132	11 - 23	(16.5)	0		12 - 30	(2.6)	0		0		90 - 96	(93.0)
Aru	28	0		0		0		0		41 - 58	(49.4)	0	
Berau Islands	29	0		0		0		0		0 - 17	(5.8)	0	
SE Sabah	30	0		0		0		0		0		0	
Sulu Sea	67	0		0		0		4 - 10	(6.7)	0 - 6	(1.0)	0	
Sarawak	22	0		0 - 3	(1.5)	5 - 10	(7.2)	0		0		0	
Peninsular Malaysia	27	30 - 54	(41.7)	0 - 10	(1.4)	0		0		0		0	
Ashmore Reef	20	0		0		0		9 - 24	(16.4)	0		0	
Scott Reef	19	19 - 37	(28.0)	1 - 38	(19.3)	9 - 25	(17.1)	12 - 34	(23.0)	0		0	
West Java	23	0		0 - 18	(9.0)	0 - 3	(1.1)	0 - 9	(3.9)	0 - 9	(1.1)	0	
North West Shelf	45	3 - 19	(11.0)	48 - 70	(59.7)	26 - 43	(34.8)	23 - 39	(31.1)	3 - 12	(7.8)	0	

[1] Note that the results for CK were considered unreliable due to substantial differences with the Conditional Maximum Likelihood model

Discussion

Spatial structure and connectivity

This first mixed stock analysis of green turtle feeding ground compositions across an east-west transect in the Indo-Pacific suggests a complex network of connectivity among nesting and feeding habitat in this region. Different stocks are not randomly distributed across the available feeding grounds, as indicated by the observed genetic structure among the feeding grounds. In a pattern of diffuse migratory connectivity, individuals from a single stock migrate to several different feeding grounds. Additionally, nearly all feeding grounds were shown to contain multiple stocks, and include adjacent or neighbouring stocks (e.g. SEP-GoC, AI-ARU) at < 500 km but also stocks at > 500 km distant to the feeding ground. Despite these varied patterns, a relationship emerged to indicate that contribution to a feeding ground in part depends on the stock's proximity, but not on its size.

Mark-recapture and satellite telemetry studies provide valuable information about movements of individual turtles that can be used in conjunction with genetic data to reveal the magnitude and complexity of the migratory connectivity within a geographic region. For example, marked turtles from both the NW Shelf and nGBR stocks have been captured at the Aru feeding grounds in Indonesia (Prince 1993; Schulz 1996; KD pers. obs.; Limpus *et al.* 2003) and the mixed stock analysis infers that these stocks each make modest contributions to this feeding ground. Tag-recovery data from throughout the region are consistent with the finding that most feeding grounds are comprised of significant contributions from multiple stocks (e.g. De Silva 1986; Prince 1997; Limpus *et al.* 2005). Importantly, the modelling output that indicated a relationship in which stock contribution to feeding grounds decreases with distance is also consistent with the more extensive tag-recovery data in eastern Australia. Long-term studies in the GBR suggest that although tag-recovery data are widely dispersed, the majority of turtles nesting at the nGBR and sGBR stocks use feeding grounds within about 500 km of their respective rookeries (Limpus *et al.* 2003; Limpus *et al.* 2005).

In addition to tag-recovery data, the genetic analysis is supported by various satellite telemetry studies on green turtles in the region. In particular, all of the 25 post-nesting turtles that were tracked by satellite telemetry from their nesting beaches within the western GoC stock migrated approximately 150 km south to their residential feeding ground at SEP within the Gulf (Kennett *et al.* 2004). Again, these data support the notion that feeding grounds in the south-western GoC are comprised primarily of GoC nesters. Migrations beyond the nearest feeding grounds were demonstrated by a green turtle nesting on Ashmore Reef that was tracked travelling to the Tiwi Islands, adjacent to CP, upon completion of her breeding

season (Spring 1994; Spring & Pike 1998) and green turtles from the Scott Reef stock were tracked to Cobourg Peninsula (R.I.T. Prince pers. comm.). In Indonesia, one of three green turtles receiving a satellite transmitter at the rookery on Piai Island (West Papua), travelled to the Aru feeding grounds (at approximately 1000 kms) and remained there (Gearheart 2005), as did two individuals that received a transmitter while nesting at one of the Palau Islands, Micronesia (at 1500 kms, Klain *et al.* 2007). Preliminary genetic characterisation of the Piai Island nesting population (Velez-Zuazo pers. comm.) indicates a grouping with the PNG stock, consistent with the contribution of this stock to the Aru feeding ground. Other satellite telemetry studies have found various post-nesting migration distances. The largest mean distance travelled (1968 km) was reported for individuals from Ascension Island tracked by satellite while migrating to residential feeding habitat along the Brazilian coast (Luschi *et al.* 1998). Green turtles from WanAn Island in the east China sea travelled on average 687 km (Cheng 2000) and from the Tortuguero rookery in Costa Rica 512 km (Troeng *et al.* 2005) to their respective feeding grounds.

Uncertainty

The ranges of possible proportional contributions to a mixture within a 90% confidence interval are broad for most of the studied feeding grounds and the results in this study only allow broad inferences on migratory connectivity among nesting and foraging habitat. The uncertainty can be attributed any one or combination of the following limitations 1) reduced analytical power associated with shared common haplotypes, 2) the use of a single molecular marker and 3) relative small sample sizes of both the contributing and the mixture populations 4) presence of novel haplotypes. In the following paragraphs we discuss these limitations.

The presence of a large diversity of haplotypes (Dethmers *et al.* 2006), but with very few regionally diagnostic ones, and some that are widely distributed (C1 and C3), reduces the ability to differentiate among the feeding aggregations and nesting populations. This is especially a problem in the eastern Indian Ocean, where there is low among-stock divergence in mtDNA haplotype frequencies. By contrast, information content of mtDNA is much higher across the central and eastern Sahul Shelf because of major frequency shifts, manifest as high frequency private alleles in the sGBR, nGBR, GoC, and Aru breeding populations (Dethmers *et al.* 2006). To better understand the missing links, future genetic analyses will need to increase the sampling effort, and should aim to increase the power of analyses by employing more complex, hierarchical Bayesian models (e.g. HWLER, Pella & Masuda 2006).

Unfortunately, preliminary analyses of microsatellite data from two of the feeding grounds, SEP and FI , using assignment tests produced very low levels of assignment to the nesting

populations (McCann *et al.*, unpubl. data). This suggests a limited usefulness of microsatellite data to analyse feeding ground compositions for these populations, which we suspect is due to relatively low levels of differentiation among nesting populations (FitzSimmons *et al.* 1997) and homoplasy across this broad geographic scale.

Incomplete sampling of nesting populations in some areas may have biased the results, particularly by reducing the capacity to detect contributions from more distant rookeries. The region encompassing the stocks included in this study covers a vast expanse of habitats suitable for feeding turtles, as well as a widespread distribution of nesting activity. Sampling and genetic characterisation of breeding populations is strong across western, northern and eastern Australia, Borneo and the eastern Sunda Shelf, in comparison to the northern Indian Ocean, south China Sea, southern Sunda Shelf, Papua and the adjacent western Northern Pacific Ocean. However, much of the nesting activity in these areas is currently at low density, and some areas influenced by recent, severe population declines (Limpus 1997). Thus, limited sampling of rookeries in these areas could confound mixed stock estimates, particularly for the Cocos Keeling Islands, Aru and Ashmore Reef foraging aggregations. Small samples sizes for some of the sampled stocks would have precluded the detection of haplotypes at low frequencies and may also have contributed a bias to the results. However, recent analysis of an additional 21 samples collected at the Ashmore Reef nesting area did not produce shifts in haplotype frequencies in space or time and as a result the estimated contributions to the feeding areas remained the same (Jensen, pers. comm.), thus confirming adequate genetic characterisation of a sampled stock in this region with a sample-size of 20.

Identifications of novel haplotypes is common in studies of mixed sea turtle stocks and range from 0.7% of observations at a single feeding area (Bass *et al.* 2004) to 5% of observations at two feeding areas (Roberts *et al.* 2005). While novel haplotypes are rare, the contributing stocks that they represent are not necessarily rare. Such haplotypes could possibly reflect remaining individuals of one or several severely depleted stocks, which are influencing the analysis. For example, the AR stock was heavily exploited prior to the declaration of the Ashmore Reef National Nature Reserve in 1983 (Russell 2005). Ashmore Reef is a small stock that had an estimated marginal representation (9 - 24%) at only the Cobourg Peninsula feeding ground. However, a tag recovery from the AR stock at Weipa, in the eastern GoC (2,050 km distant, QTC turtle research database) well beyond Cobourg Peninsula indicates the need for further investigation of the feeding range for this stock. Further research into the origin of these unidentified contributions would not only improve our evaluation of the foraging aggregations but would also provide insight into the status of some breeding stocks.

Migration and dispersal

The constraints have limited our ability to draw robust conclusions and emphasized the importance of the use of both genetic and field-based methodologies to better understand the origins of turtles at feeding grounds and reveal the magnitude and complexity of migratory connectivity within a geographic region. We have shown that the relationship between distance and contribution is particularly strong for the SEP and Aru feeding grounds. In this analysis, the south-western GoC feeding ground (SEP) is dominated by GoC nesting turtles (from < 500km distance), with a relatively small contribution from nGBR (from ~1,000 km distance) and a negligible proportion of turtles coming from sGBR (from ~2,500 km distance). This result supports the tag-recovery data. Of the tens of thousands of nesting females tagged at the nGBR and sGBR stocks, 12 and 3 respectively have been recaptured at SEP; while two migrants from the few thousand tagged while nesting within the GoC stock have been recaptured there (Limpus *et al.* 2003; C. Limpus, unpublished data from the Queensland Turtle Research database). At Aru, the genetic conclusions appear robust given that they are largely based on high frequency (86%) of a haplotype (C14) that is found in 96% of the Aru nesting turtles, as compared to 29% at the Berau stock and < 10% in some Malaysian and Australian stocks (Dethmers *et al.* 2006). However, Schulz (1996) reported a recapture at the Aru feeding grounds from a turtle nesting within the Sulu Sea stock, and this stock was not detected at a significant level in the genetic analysis due to large standard errors. In the north Atlantic region, the relative importance of distance and population size of stocks to their contribution appears to vary among feeding grounds and species. Lahanas *et al.* (1998) found that the size of green turtle populations was a strong predictor of estimated contributions to a feeding ground in the Bahamas, but the influence of distance was insignificant. Contributions of hawksbill turtle (*Eretmochelys imbricata*) populations to various feeding grounds in the Caribbean were significantly correlated to both factors (Bowen *et al.* 2007), but neither of these factors correlated in green turtle contributions to a Barbados feeding ground (Luke *et al.* 2004).

In theory, the connection between the breeding sites and the foraging areas is established via the oceanic pelagic dispersal of the small post-hatchling green turtles from their respective natal beaches via ocean currents (Bolten 2003). However, the details regarding the temporal and spatial distribution of the post-hatchlings as they move with the currents is poorly understood, especially for the stocks of northern and western Australia (Walker 1990). Even less is known regarding the age and size structure and behaviour of the large post-hatchlings as they return to coastal waters and recruit to the benthic foraging populations of the region within this present study. This study demonstrates that small immature turtles from the one stock can recruit to multiple foraging areas within several thousand kilometres of a breeding

area. The gyre of the Arafura Sea - Gulf of Carpentaria (Wolanski 1993), and the Indonesian Throughflow (Verschell *et al.* 1995; Bray *et al.* 1996) have a likely influence on the dispersal patterns of individuals from the nGBR, sGBR and NW Shelf stocks within feeding sites of the Sahul Shelf and Gulf of Carpentaria. While these relationships are not obvious at this time, this study suggests that individuals from the NW Shelf and Scott Reef stocks have recruited in feeding areas to the north east of the breeding sites and apparently against the predominant currents (Verschell *et al.* 1995; Bray *et al.* 1996).

Conclusion

The methodology explored in the present study provides a broad indication of stocks that are represented in a feeding aggregation and thus a preliminary insight into the potential geographic extent of the impact associated with anthropogenic mortalities. For example, a high level of mortality at Cobourg Peninsula could negatively impact on multiple breeding populations, whereas a similar level of mortality in the Sir Edward Pellew Islands is likely to primarily impact the Gulf of Carpentaria stock. Similarly, the NorthWest Shelf and Aru stocks can be expected to be heavily impacted by exploitation pressure at the Ashmore and Aru feeding grounds respectively. Although the results at this stage do not provide a solid basis for firm management decisions, management can be guided to focus on obtaining missing data and information on the stocks of concern. Ultimately, the severity of the impact or the success of potential management actions depends on three variables: 1) the level of representation of each of the stocks at a single feeding ground; 2) the total population size of affected stocks and 3) the distribution of affected stocks over (potentially) multiple feeding grounds.

The southern Gulf of Carpentaria presents an interesting management scenario for green turtles, as the data indicate that few individuals from stocks other than the GoC stock migrate into the southwestern Gulf. While tagged individuals from the GBR stocks have been recaptured within the GoC, the genetic analysis show that their overall contribution to the SEP feeding aggregation is estimated to be proportionally small (4.2 ± 3.0%) for the nGBR (from ~1000km) and undistinguishable for the sGBR (from ~2500 km). With the GoC stock estimated to be almost the sole contributor of the SEP aggregation, a potential reduction of the foraging aggregation such as the through the loss of sea grass habitats as reported by Indigenous hunters in the region (Kennett pers. comm.), or cyclones (Limpus & Reed 1985a) would have a direct impact on the GoC stock. Any decline in the feeding aggregation would be poorly compensated for due to minimal recruitment from other stocks. Conversely, management actions directed at green turtles within the Gulf of Carpentaria have a greater chance of success as the stock is less affected by other unmanaged impacts outside the Gulf of

Carpentaria (Kennett *et al.* 2004). This scenario is well suited for coordinated management actions focussed on both rookeries and feeding grounds as is currently being planned and implemented by Aboriginal organisations within the Gulf of Carpentaria.

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5.

Extinction risk analysis of exploited green turtle (Chelonia mydas) stocks in the Indo-Pacific

K.E.M. Dethmers and P.W.J. Baxter

Animal Ecology - Submitted

Abstract

Although the green turtle (*Chelonia mydas*) is listed as a globally endangered species, local conservation successes have led to suggestions that this species is not really threatened with extinction. However, anthropogenic induced mortality continues to affect populations in many regions worldwide causing rapid local and regional declines. Thus, while on a global scale green turtles may persist, on a regional scale the species could face extinction without intervention. For the majority of sites throughout the Indo-Pacific region, a paucity of key demographic data has hindered accurate estimation of the probability of local extinctions to determine conservation status regionally and to support management decision-making. We used demographic and genetic data in an age-based model to investigate the viability of exploited green turtle stocks in the Indo-Pacific region. Based on best available data this model provides the most parsimonious approach to determining stock persistence. We found that populations are decreasing under current exploitation pressures. Effects of increasingly severe exploitation activities at foraging and nesting habitat varied depending on the migratory patterns of the stock. Our model predicted a rapid decline of the Aru stock in Indonesia under local exploitation pressure and a shift in the genetic composition of the stock. We used the model to test the effectiveness of different conservation actions. The results show that local management actions such as reducing mortality of adult nesting females and enforcing local harvest quota are sufficient to ensure the long-term persistence of genetically distinct management units.

Introduction

Marine turtles have long been exploited for human subsistence. Indigenous coastal communities in many regions relied on marine turtles as a source of protein (Balazs 1983; Barraud 1990) and continue to do so. Harvest rates were generally confined to particular near-shore marine territories and limited by the use of primitive vessels and equipment. However, harvest rates increased dramatically following European colonization events (Jackson *et al.* 2001), which brought technological advances to previously isolated regions as well as transitions to a market driven economy. Previously undisturbed populations became heavily exploited, sometimes to near extinction (Limpus *et al.* 1994; Bjorndal *et al.* 2000; McClenachan *et al.* 2006). Although recent conservation efforts have resulted in increasing population sizes in some regions (see NMFS 2007 for an overview), large-scale turtle exploitation continues throughout parts of the Indo-Pacific. Declining populations (Limpus 1997) and at least one reported extinct population (Chan & Liew 1996) in this region indicate that marine turtle populations in the Indo-Pacific may not persist under the current level of anthropogenic mortality.

Current debate is arguing whether the green turtle (*Chelonia mydas* (Linnaeus 1758)) is correctly listed as a globally endangered species on the IUCN red list (IUCN Species Survival Commission 2007). Several populations within the Atlantic region have more than doubled in size since management intervention was put in place, e.g. in Costa Rica (Bjorndal *et al.* 1999), Florida (Meylan *et al.* 2006), and Ascension Island (Broderick *et al.* 2006). As such, it has been suggested that the Atlantic green turtle populations may no longer require to be listed as endangered (Broderick *et al.* 2006). By contrast, in the Indo-Pacific region little is known about the status of most populations. Some populations in Australia and Malaysia increased (Chaloupka & Limpus 2001; De Silva 1986), but other populations decreased, e.g. in Vietnam (Hamann *et al.* 2006), Thailand (National Marine Fisheries Service & U.S.Fish and Wildlife Service 2007) and parts of Indonesia (Schulz 1989; Limpus 1997; Dethmers *et al.* in prep. a). However, for the majority of the many other sites throughout the region, the paucity of data impedes accurate estimation of conservation status and anthropogenic impact.

The southeast Indonesian Aru archipelago presents such a scenario. It once had large populations of several marine turtle species (Compost 1980; Schulz 1996) nesting at beaches and foraging at the seagrass beds. The people of Aru traditionally harvested turtles for subsistence and ceremonial use. Starting in the mid 1980's an increased commercial value of meat from green turtles in other parts of the country dramatically increased the take of this species both from nesting beaches and foraging grounds. The long-term persistence of green

turtles under the continuing commercial harvest remained unclear because of this species' long-distance migratory behaviour, and late maturation. Consequently, no management plan was developed to ensure the continuity of traditional turtle harvesting.

The persistence of a population under influence of harvest depends in large part on the life history strategy of the target organism (Heppell *et al.* 2000) on the relative intensity, selectivity and timing of the harvest; on additional human-imposed mortality (Hunter & Caswell 2005) and on the dispersal capacity of the organism. Stage- and age structured population models are widely used as a tool to assess impact of harvest and, used with care, can aid conservation decisions (Thompson *et al.* 2000; Akcakaya & Sjogren-Gulve 2000; Maehr *et al.* 2002; Baxter *et al.* 2006). For threatened species assessment, matrix population models can provide an informed understanding of the species' viability under the influence of threatening processes. Since matrix models were first successfully applied to marine turtle conservation (Crouse *et al.* 1987) they have been used to assess the sustainability of marine turtle harvesting in both the Atlantic and Pacific regions (Heppell *et al.* 1996; Heppell & Crowder 1996) and have become the underpinning foundation of population viability analysis (PVA; Beissinger & Westphal 1998). Crucial for developing such models are key demographic parameters, either measured directly or, when not available, inferred from geographically proximate populations.

Incorporation of genetic information has the potential to guide management decision-making at a broader geographic scale. Genetic analysis of green turtles nesting in Aru identified this population as a distinct stock (Dethmers *et al.* 2006). Further analyses based on mtDNA sequence divergence and haplotype frequency variation revealed that around 50% of the foraging aggregation in Aru is composed of the Aru stock (Dethmers *et al.* in prep. b). At least three other genetic stocks that migrate to remote nesting habitats share these feeding grounds with the Aru stock (Figure 5.1). Our aim in this study is to use genetic information in combination with population demographic data to assess the effect of continuous harvesting on the persistence of green turtle stocks in Aru. Furthermore, we investigate how various threat scenarios, both locally and remotely, affect local and remote genetic stocks. We then assess how different possible management strategies could influence population trajectories and prevent possible extinction both locally and regionally.

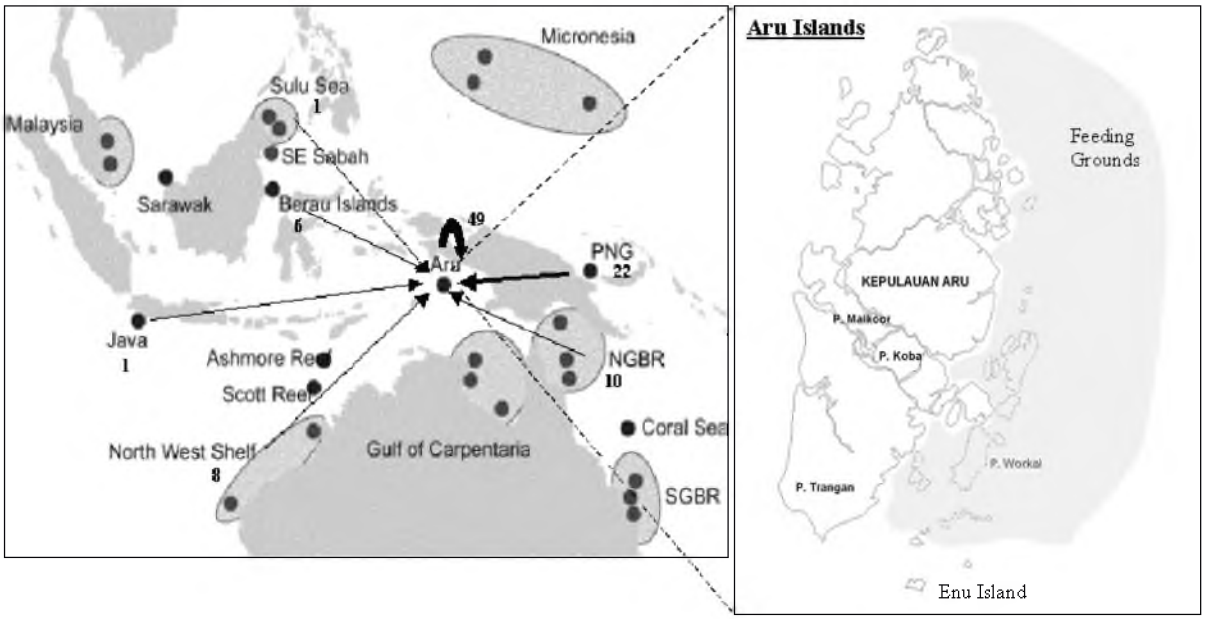


Figure 5.1. Location of the Aru archipelago within Australasia (central point in left panel), the Aru feeding grounds (shaded area in right panel) and Enu Island rookery. The arrows indicate migratory links between various genetically distinct breeding stocks (MUs; dots in the left panel) and the Aru feeding ground.

Methods

Population dynamics and model structure

We divided the Australasian green turtle population into four subpopulations (Figure 5.2) for individuals that: nest on the main nesting beach (Enu Island), and remain at the feeding grounds within the Aru archipelago during the interbreeding interval (EA); migrate to nest on Enu Island from remote feeding areas (ER); migrate to remote nesting habitat from the Aru feeding grounds (RA); and those that nest and feed remotely (RR). The 'Aru stock' refers to all individuals that return to Enu Island each breeding cycle (EA + ER). The 'Aru population' refers to all individuals that can be observed within the Aru archipelago throughout most of their life cycle (EA + RA). Furthermore, to describe the links between these four subpopulations, we employed three parameters (Figure 5.2). The subdivision of EA and ER is given by α, and β denotes the proportion of the foraging aggregation that nests on Enu Island. ϕ denotes the proportional size of the Aru foraging aggregation relative to the total foraging area within the Australasian region.

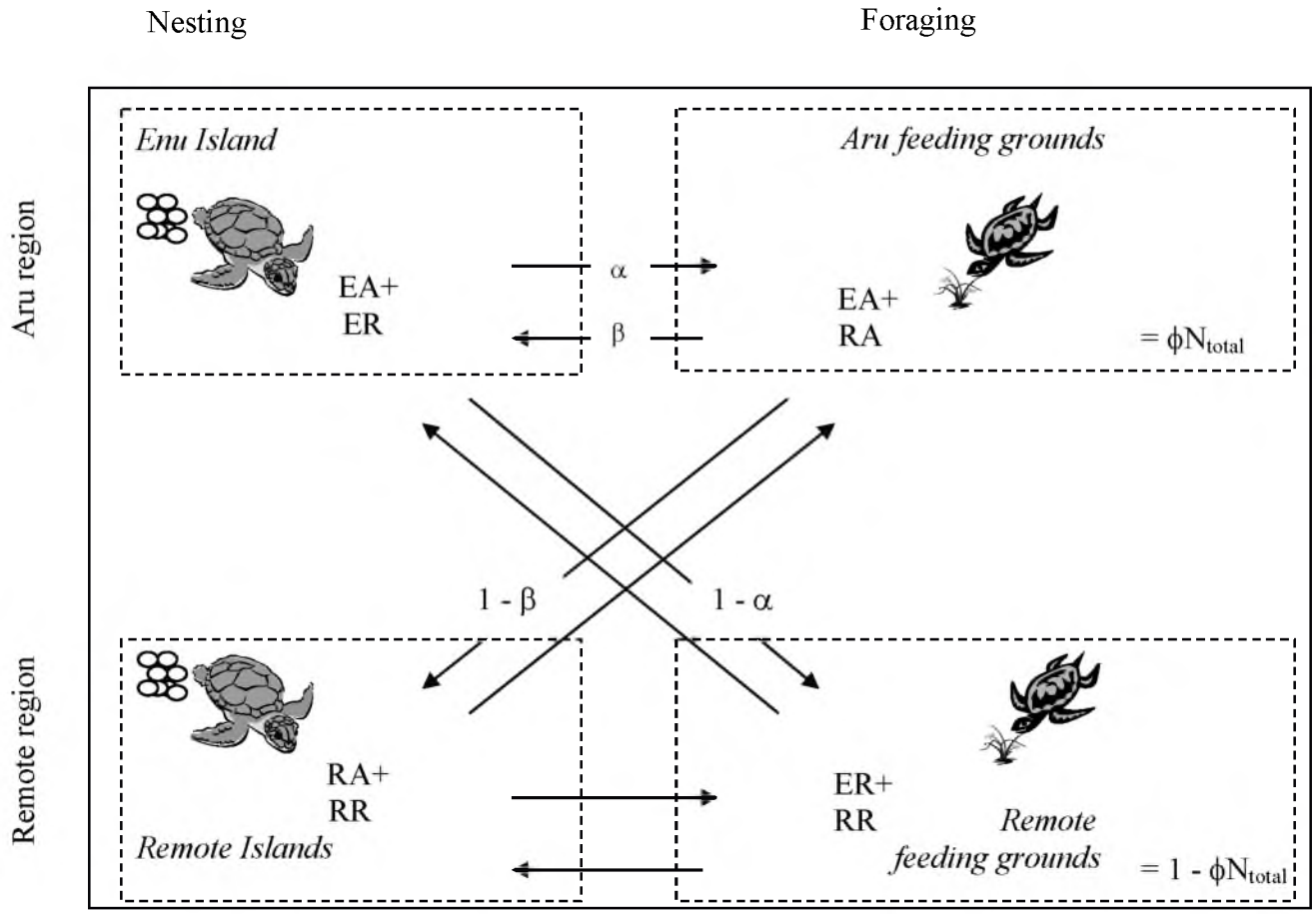


Figure 5.2. Model structure of *Chelonia mydas* in the Aru archipelago, with the four subpopulations comprising the proportion of turtles that nests on Enu Island and forages at the Aru feeding grounds (EA), or remotely (ER), and the proportion of turtles that nests remotely and forages at Aru (RA), or remotely (RR). Links between the subpopulations are described by the proportion of Enu nesters that forages at the Aru feeding grounds (α); the proportion of the Aru feeding aggregation that nests on Enu Island (β); and the relative size of the Aru feeding ground in comparison with all other accessible but remote feeding grounds (ϕ).

The total population of Australasian adult females is defined as N_{reg}. We set the initial Aru stock as EA + ER = E = 5500, giving EA = αE; ER = E(1 – α); RA = αE(1 – β)/β and RR = E(α + $\alpha/\beta\phi$ – (1 + α/β)) (Figure 5.2), constraining $\alpha \geq \phi$ to ensure RR ≥ 0.

We structured the population into 60 age-classes ($0 \leq x \leq 59$), assuming green turtles have a lifespan of approximately 60 years (Chaloupka & Limpus 2005); however we also allowed survival within the final age-class. In our model we considered females only and assumed that

fecundity and survival rates are the same for each subpopulation. Each subpopulation's dynamics is then described by:

$$\mathbf{n}_{ij}(t+1) = \mathbf{A}(t)(\mathbf{n}_{ij}(t) - \mathbf{h}_{ij}(t)),$$

where $\mathbf{n}_{ij}(t)$ and $\mathbf{h}_{ij}(t)$ are the subpopulation and harvest vectors, respectively, of turtles breeding at site i (i = E, R) and feeding at j (j = A, R) at time t. $\mathbf{A}(t)$ is the 60 x 60 Leslie matrix describing the population dynamics, which was stochastically generated at each time t. The subdiagonal elements of $\mathbf{A}(t)$ contain age-specific survival rates s_x and the first row contains age-specific fecundity rates f_x; all other elements are zero. The fecundity rates are the product of number of clutches laid per female per season, number of eggs per clutch, and hatching success.

Population demography

Based on census data over six years (Chapter 2) the total breeding population of Enu Island was estimated at 5500 females. Population demographic data of the nesting population were collected from 1997-2000 (unpublished data). Based on mitochondrial DNA analysis, the population nesting at Enu is a genetically distinct stock (Dethmers *et al.* 2006). The composition of the foraging aggregation was determined through mixed stock analysis of 40 tissue samples. The analysis uses a pseudo-Bayesian maximum likelihood model (Pella & Masuda 2001) that compares haplotype frequencies and sequence variation among the feeding ground samples with that of 17 potentially contributing genetic stocks, including the Aru stock. The model estimated that an average of 50% (range from 0.25 – 0.82 in 1000 iterations) of the feeding aggregation are turtles that nest on Enu Island (β), while the other 50% migrate to remote nesting habitat (Dethmers *et al.* in prep. b; see Figure 5.1).

Demographic parameters

As in many studies on long-lived vertebrates, detailed field data, which are needed to determine population-specific demographic parameters, are scarce for the Aru stock. Table 5.1 summarizes the demographic parameters collected on the Enu nesting population, and those extrapolated from the geographically and phylogenetically most proximate population from the Great Barrier Reef green turtle populations. Yearly survival rates for each age, year and run, were generated through random sampling from beta distributions estimated in the literature (Chaloupka & Limpus 2005). Intrinsic fluctuations in annual green turtle reproduction (e.g. Limpus *et al.* 2003) were synchronized for all subpopulations through random selection of the variables by year. We varied age at maturity and remigration interval

triangularly across integer values only, for each run. For number of clutches per female per breeding season, eggs per clutch and clutch success rate, we selected a uniform [0, 1] random number and back-calculated the relevant value based on recorded cumulative distributions for Raine Island, Northern Great Barrier Reef (clutches/female/season); and for Enu (eggs/clutch and clutch success rate; Dethmers *et al.* in prep. a). For β we again selected a uniform [0, 1] random number for each simulation and back-calculated the relevant proportion based on the maximum likelihood distribution. We assumed that other stocks in the region have similar population demographics.

Harvest

Our primary harvest data represent the catches taken at the Aru feeding grounds by non-resident turtle hunters. We estimated the total number of ships operating in Aru based on interviews with various stakeholder groups (village elders, fishers, and the departments of Fisheries and Nature Conservation), and registered the annual number of return trips an average ship makes to their home market. Demographic data for the foraging population were collected during anti-harvesting patrols carried out by the Indonesian Department of Nature Conservation (KSDA) in 1998 and 2000. In total, 154 captured foraging turtles were measured and marked, of which 40 individuals were randomly sampled for genetic analysis. Approximately 5000 turtles are harvested from the Aru feeding grounds annually (Dethmers 2000). Sex ratio was based on a detailed gonad study of 170 green turtles landed at Tanjung Benoa harbour (Bali) that determined 67% of the landed individuals were females (Adnyana 1997. The harvest rates were distributed triangularly across [3015, 3685] (i.e. 0.67[5000] ± 10%), for each year in each run. We assumed that the harvesting vector was size-dependent, with fishers more likely to take turtles according to their relative size and abundance. Chaloupka *et al.* 2004) show age-specific growth curves for green turtle populations in sGBR, which we approximated here by the equation:

$$g_x = 0.5xe^{-0.08x},$$

where g_x = growth at age x (calculated only for harvestable turtles, i.e. $x \geq 5$) in cm-CCL. By setting an initial size for turtles arriving at the feeding grounds (i.e., entering the harvestable population) we could calculate all sizes c_x (= $c_{x-1} + g_{x-1}$). Assuming that turtles at age 5 are of size c_5 = 40 cm-CCL (Limpus *et al.* 1994) produces a size distribution which approximates those of Chaloupka *et al.* (2004). We assume that the subpopulations at feeding ground j are well mixed so that the age-specific harvest $h_{ij,x}$ for subpopulation N_{ij} is a weighted average of overall abundance and size:

$$h_{ij,x} = H_j \frac{N_{ij,x} c_x}{\sum_{i=E,B} \left(\sum_{x=5}^{80} N_{ij,x} c_x \right)},$$

where H_j is the total harvest (of females) at feeding ground j.

Model scenarios

There is limited information available for assessing the impact of all possible sources of anthropogenic mortality in the region. Our null scenario of local harvest-impact only is therefore rather optimistic and should be interpreted with caution. In our null scenario, we estimated the probability of persistence of the Aru stock (E) over a period of 100 years, with initial population size of 5500, and expressed in number of females. This scenario assumes that the proportion of green turtles that nests on Enu Island and forages in Aru is equal to the proportion that migrates to distant feeding grounds ($\alpha = 0.5$), and also that the proportion of green turtles foraging at the Aru feeding grounds is equal to those foraging at the combined remote foraging areas ($\phi = 0.5$). Spatial variability of threats (e.g. harvesting at other feeding grounds, by-catch along migration routes, predation etc.) is ignored. Subsequently we determined the trajectories for the probabilities of persistence of all four subpopulations, centred on the Aru MU and harvest regime at the Aru feeding grounds. To investigate the relative influence of α and ϕ, we repeated the initial scenario with (α, ϕ) = (0.25, 0.25), (0.50, 0.25), (0.75, 0.50) and (0.75, 0.75). To reduce transitory effects from the initial population structure in our model, we set the initial population structure for each subpopulation and scenario to the mean structure (from 1000 simulations) of that subpopulation after 100 years of simulation under that scenario.

As exploitation does not only occur at the Aru feeding grounds, we investigated the probability of extinction ($N < 1$) and quasi-extinction ($N \leq 50$ and $N \leq 500$) under increasing threat levels over the next 200 years. We defined the following scenarios: A = harvesting at Aru feeding grounds and a 50% take of females off the Enu rookery; B = as A but including a random 10-100% egg-take at the Enu rookery; C = as B and including harvesting at remote feeding grounds at a similar rate as on Aru; D = as C with a 50% take of females and 10-100% egg-take at remote rookeries.

Finally we assessed the effect of a series of management interventions implemented at the worst scenario considered (Scenario D), starting at a local and easily realizable scale. The most feasible management strategy is usually some level of nest protection, allowing an increase in hatching rates. An additional step is to reduce the number of adult females taken

from the rookery. Next would be to impose a harvest quota locally, and ultimately also on a regional or international level through collaboration in multi-lateral management agreements. When implementing local management strategies, we allowed a continued remote exploitation scenarios of 10 – 100% egg collection, 50% nesting female mortality and around 3000 adult females harvested at remote feeding grounds. For region-wide management we let all local and remote strategies be similar. In assessing the effect of harvest quota we let 500 turtles be the minimum number of individuals harvested per year to allow for other types of anthropogenic mortality, such as by-catch.

Table 5.1. Demographic parameters for *Chelonia mydas* for model development

Population parameters	Parameter estimates	Stochasticity	Unit	Source*	Comment
Effective pop. size (N_{af}) of Aru stock	5500		Females	1	Deterministic
Age at first recruitment to FG	5		Years	2	First recruit min. CCL = 40 cm
Growth rate	0 - 2.5	0 - 2.5	cm CCL/yr	2	Approximation of sGBR study
Life span	60		Years	3	
Hatchling survival [x = 0]	0.4394	sd = 0.0400		4	Beta distribution (now)
Pelagic-stage survival [0 < x ≤ 4]	0.6445	sd = 0.0050		4	Beta distribution
Juveniles survival [4 < x ≤ 18]	0.8804	sd = 0.0234		3	Beta distribution
Subadults survival [18 < x < age at maturity]	0.8474	sd = 0.0302		3	Beta distribution
Mature adults survival [x ≥ age at maturity]	0.9482	sd = 0.0151		3	Beta distribution
Age at maturity	31.5	28 - 35	Years	2 and 5	Triangularly distributed **
Remigration interval	mean is 5	[4,6]	Years	6	Triangularly distributed **
Clutches / ♀ / season	mean is 6.2	range is [1, 10]	Number	7	Sampled from distribution
Eggs / clutch / ♀	106	46 – 159	Number	8	Sampled from distribution
Hatching success rate	82.2	48 – 100	%	8	Sampled from distribution
β (prop. Aru feeders nesting on Enu)	0.5	0.25 – 0.82		9	Sampled from distribution
Aru harvest	3,500	[0.9, 1.1] x input rate	Females	8 and 10	Triangularly distributed
Remote harvest	3,500	[0.9, 1.1] x input rate	Females		Independent of Aru

* 1) Dethmers *et al.* 2006; 2) Limpus and Chaloupka 1997; 3) Chaloupka and Limpus 2005; 4) Chaloupka 2002; 5) Limpus 1992; 6) Limpus *et al.* 2003; 7) Limpus *et al.* 2001; 8) KEMD; 9) Dethmers *et al.* 2007; 10) Adnyana 1997

** Integer values only

Results

Population dynamics

Simulations in our null model predict that the Aru stock (EA+ER) and population (EA+RA) will persist and even increase under an annual harvest of approximately 3000 females taken from the feeding grounds in Aru for the next 50 years (Figure 5.3a). This assumes an absence of density-dependent compensatory strategies and no other types of anthropogenic mortality affecting the stock. However, the composition of both the Aru stock and population changes: the proportion of nesters remaining in Aru decreases over time (from 50 to 40%), while the proportion of long-distance migrants increases (Figure 5.3b), with this rate of change depending on the relative proportion of "stayers" within the stock (α). A stock with a large proportion of stayers will take longer to consist of only migrating individuals (Figure 5.4).

Response to different levels of impact

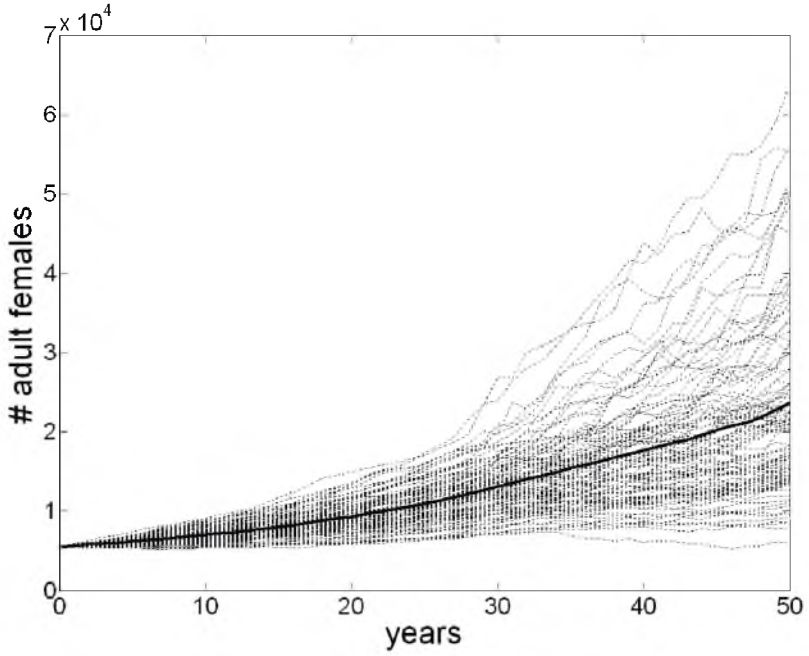
The model predicts that additional yearly exploitation of a random 10 - 100% eggs at the Enu rookery does not have a big impact on the persistence of the Aru stock, with an estimated total population size of ± 200,000 after 200 years. By comparison, the model predicted that the impact of an additional exploitation of 50% breeding females off the Enu rookery (but no egg harvest) is much more severe, with ± 24,000 individuals in the Aru stock after 200 years. Nevertheless, after 150 years of 50% breeding female exploitation, the probability of reaching the higher quasi-extinction threshold (N = 500) increases slightly (Figure 5.5 – A). However, in combination with annual random egg-harvests the probability of quasi-extinction rapidly increases after 50 years (Figure 5.5 - B). Including exploitation pressure at remote feeding grounds at a similar rate results in an earlier onset of quasi-extinction (Figure 5.5 - C). After 100 years under such conditions, quasi-extinction risks for N = 500 and N = 50 are 80% and 15% respectively, while the probability of extinction is 10%. The worst scenario considered (D), where harvesting of turtles occurs at all feeding grounds and where both adult females and eggs are taken from all nesting areas linked to Aru, is estimated to drive the Aru stock to extinction within 50 years (Figure 5.5 - D).

Effect of management interventions

Unfortunately, scenario D is the most realistic scenario and therefore the Aru stock can be expected to be extinct within 50 years. We tested the effect of several possible management strategies that might reduce extinction risk for the Aru stock under threat scenario D. Results were generated for each combination of hatching success (30 – 100%), nesting female mortality (50 – 0%) and harvest quota (500 – 5000). Protection of the nests at the Enu rookery

had little effect: with 100% nest protection the extinction risk was still 30% after 50 years and 100% after 200 years. In subsequent tests, we let hatching success be 30%. If female mortality is also reduced, extinction risk with complete protection of nests and females dropped to zero over 50 years but was 50% over 200 years (Figure 5.6 - A). A locally imposed harvest quota of 2000 turtles per year and a 50% take of female adults from Enu can be sustained for 50 years, but will have caused extinction after 200 years (Figure 5.6 - B). Probabilities of extinction for harvest quotas larger than approximately 3000 per year increased when similar quota were allowed region-wide (figures 5.6 - B and C). Interestingly, the only observed improvement of region-wide management intervention over local management could be observed for a scenario with 45% nesting female mortality and 3000 adult females harvested at feeding grounds. Here, region-wide management reduced the probability of extinction by only 2% in comparison to similar local strategies.

a)



b)

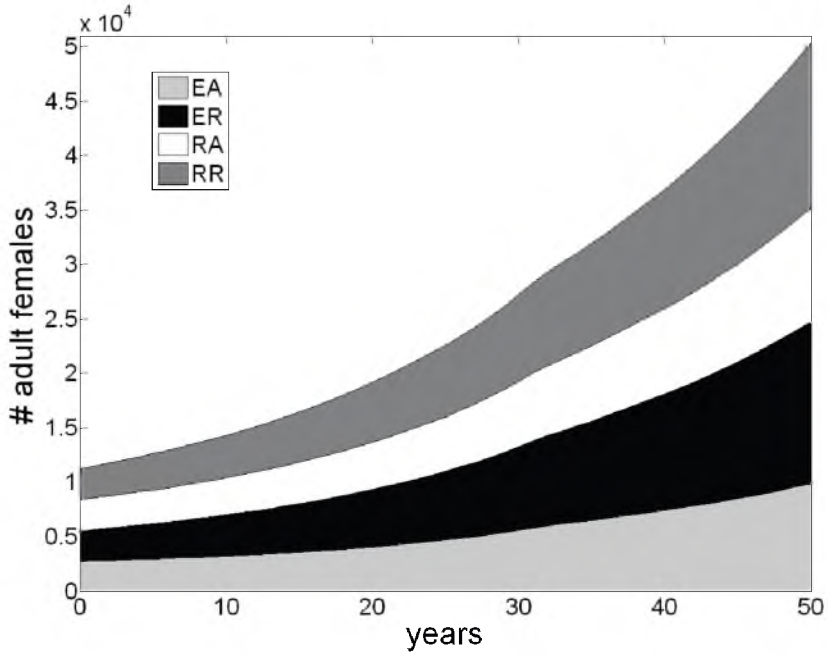


Figure 5.3. Population trajectories of an un-harvested Aru stock (EA + ER) with an initial population size 5500 (a) and equal proportions of EA and ER ($\alpha = 0.5$) and of EA and RA ($\phi = 0.5$). Results are shown of 100 replicate simulations (dotted lines) and the average of 1000 replicates (dark line). The stacked average trajectories for the separate subpopulations EA, ER, RA, and RR (1000 iterations each) with 95% CI are presented in b). (RA + RR) : (EA + ER) is determined by a random selection of the β-distribution. See Figure 5.2 for a description of the subpopulations

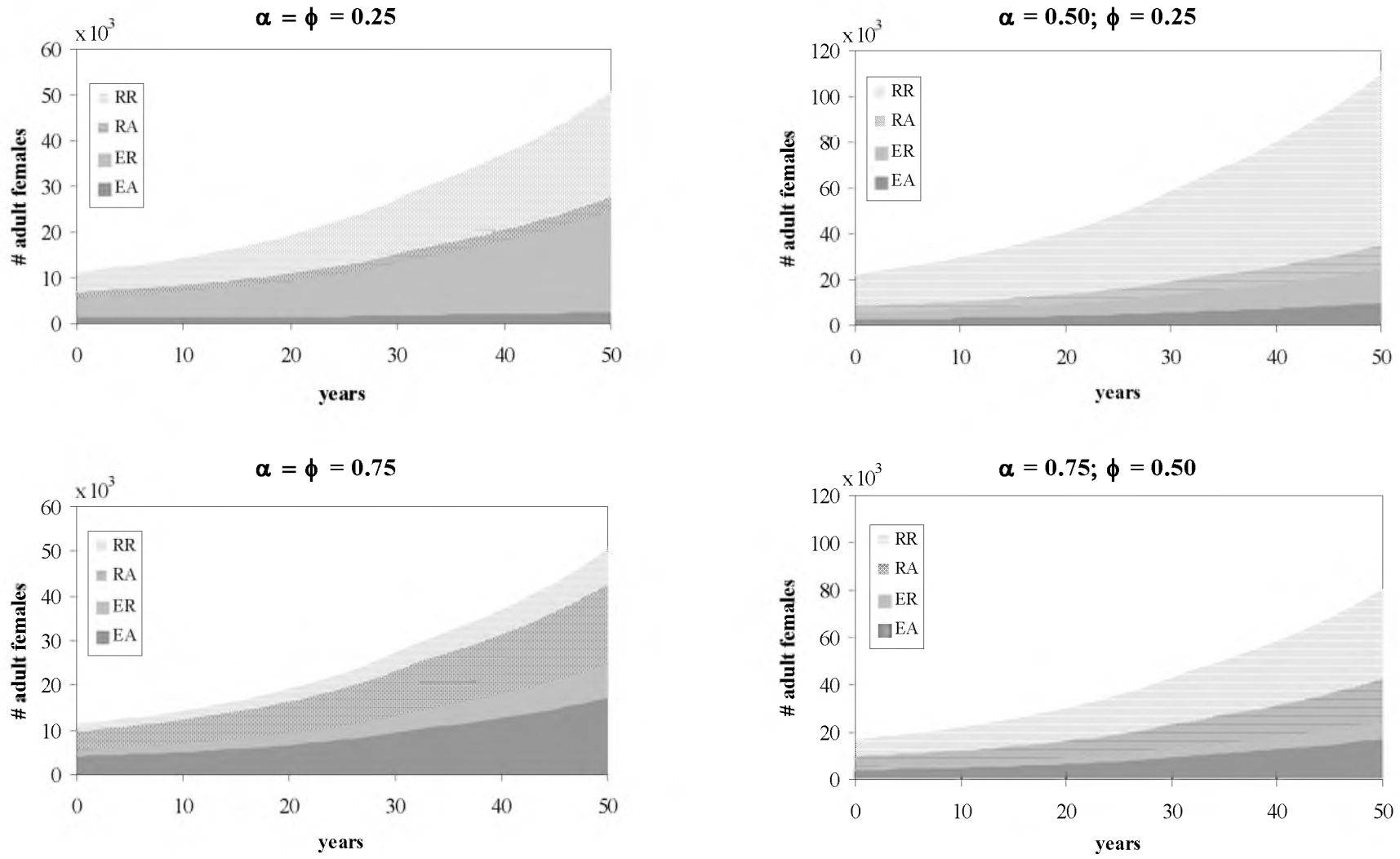


Figure 5.4. Population trajectories of all subpopulations for varying combinations of α and ϕ. Graphs in the first column represent scenarios where α = ϕ, in the second column α > ϕ. The Aru stock (EA+ER) is represented by the solid-coloured surface. Note the increased scale in the second column.

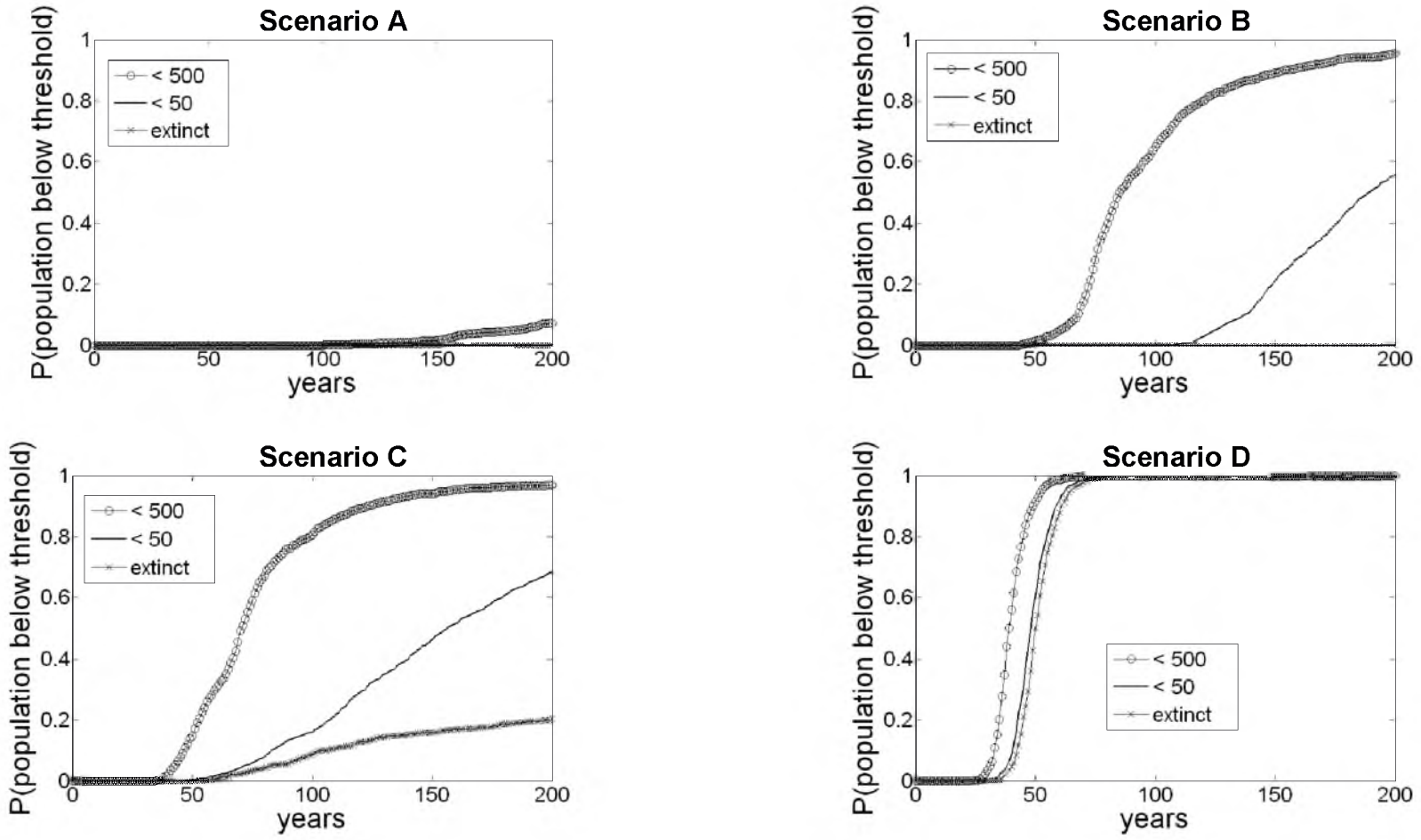


Figure 5.5. Probability of extinction (N<1) and quasi-extinction at N≤50 and N≤500 for the Aru stock in 200 years, assuming $\alpha = \phi = 0.5$ and $\beta = 0.5 \pm 0.09$ under four threat scenarios. Scenarios are as follows:

A: harvesting at Aru feeding ground AND a 50% nesting female harvest at the Enu rookery

B: as A and a random 10 – 100% egg-take at the Enu rookery

C: as B and harvesting at remote feeding grounds at a similar rate as on Aru

D: as C and a 50% nesting female turtles AND a random 10 – 100% egg-take at remote rookeries

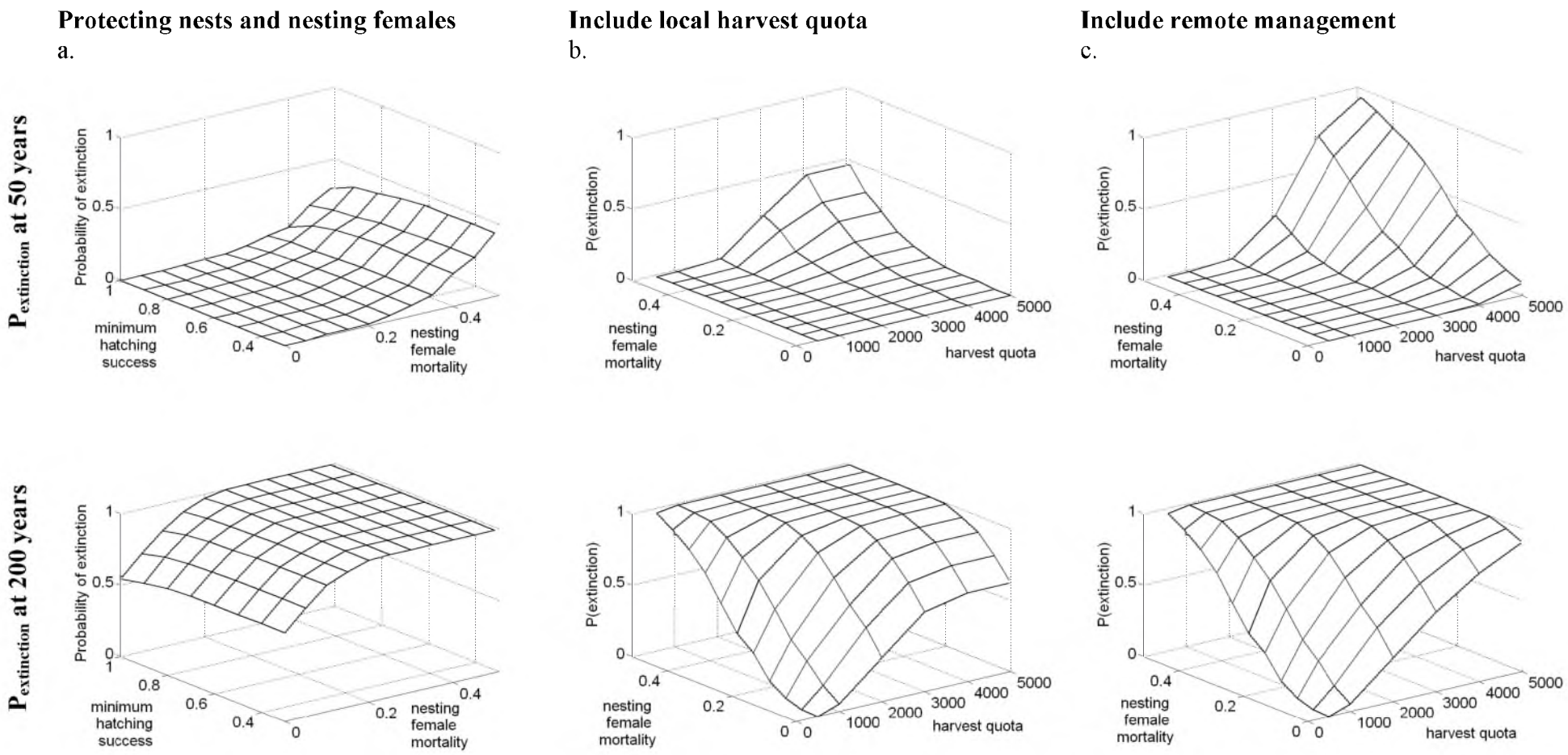


Figure 5.6. Extinction probabilities in 50 and 200 years for the exploited Aru green turtle stock (as in scenario D in Fig. 5), managed with different strategies. Strategies are as follows:

A. Protect nests and protect nesting females at Enu rookery only

B. Protect nesting females at the Enu rookery and enforce annual harvest quota at the Aru feeding grounds

C. Protect nesting females at Aru and remote rookeries and enforce annual harvest quota at the Aru and remote feeding grounds

Discussion

Continued exploitation of green turtles at feeding grounds and nesting beaches in the Indo-Pacific can drive certain stocks to extinction within 50 years. For example, egg harvest of green turtles throughout Indonesia (Wicaksono 1992; Sloan *et al.* 1994; Stringell 1999) have already greatly reduced population sizes, further exploitation pressure could jeopardize the persistence of these populations. Simulation results of our demographic model predict that the Aru stock could persist under a local fixed annual harvest but that the composition of this stock will change over the years depending on its dispersal pattern. The relative proportion of turtles that remain in Aru after the breeding season (EA) is expected to decline after 50 years, but the rate at which this occurs is dependent upon the initial proportion (α), ranging between 7% and 60% declines for $\alpha = 0.75$ and 0.25 respectively. The proportion of individuals that migrate from remote resident feeding grounds to nest on Enu Island (ER) is expected to increase over 50 years at a rate of 1.2%, regardless of α. Throughout each scenario we have maintained a constant range for the migrant versus resident ratio (β) as observed from the genetic composition data for the Aru feeding grounds. In other words, while a smaller proportion of residents implies that a smaller proportion of the Aru stock is subject to exploitation at the Aru feeding grounds, the rate of change in composition of the stock is nearly 9-fold faster.

A loss of genetic diversity has been demonstrated to reduce survival and reproduction in small populations (Frankham 1995). We expect that the composition change will be reflected by a shift and possibly reduction in haplotype frequencies. Bass *et al.* (2004) have demonstrated that haplotype frequencies in a mixed stock of juvenile loggerheads do not differ significantly between years. Possibly, anthropogenic mortality in that region is not affecting one stock more than the other. However, the observations were made across three years, a time span that is too short to be able to detect temporal changes in genetic composition as a result from anthropogenic mortality. We show that composition shifts can be expected within one turtle generation, despite persistence of the stock under a certain level of exploitation.

In the Indo-Pacific region, harvests and net entanglements are the major sources of anthropogenic mortality (Limpus & Chatto 2004). We have focused our assessment of extinction risk on the effects of direct turtle and eggs harvests, and on the Aru stock in particular. Our results show that the Aru stock is expected to persist under an annual commercial take of approximately 3000 female turtles off the feeding grounds, assuming no other anthropogenic mortality, but will eventually consist of only nesting females migrating from remote residential feeding grounds. We used a sex ratio based on turtles landed at the

Bali market. However, a proportion of these landed turtles could have been harvested from nesting beaches. This potentially inflated the actual sex ratio, which overestimates the extinction probability.

The continued long-term persistence under additional exploitation of 50% of each year's nesting cohort at Enu Island was intuitively surprising but is in agreement with other stochastic models on marine turtle population persistence (Chaloupka 2002; Mazaris *et al.* 2005). Population viability studies incorporating environmental stochasticity (Chaloupka 2002), and individual-based modeling incorporating known behavioral-ecological characteristics (Mazaris *et al.* 2005), both found that population persistence significantly depended on fertility and survival rate at early life stages, especially in the pelagic life-stage. A combined egg and nesting female exploitation scenario increased the extinction risk of the Aru stock substantially.

Such exploitation scenarios generally do not stand alone. Many indigenous communities in SE Asia and northern Australia depend on marine resources for subsistence. Subsistence harvesting of eggs and adult turtles at feeding and nesting habitat occurs throughout the region (Kowarsky 1982; Johannes & MacFarlane 1991; Suarez & Starbird 1996; Broderick 1998). Our model showed that additional mortality at remote feeding grounds at rates similar to those in Aru further increased the probabilities of extinction of the Aru stock. Here the impact affects the migratory proportion, and it clearly illustrates how exploitation activities in one region can affect the persistence of remote populations. The Aru stock is most vulnerable when, in addition to local mortalities, adult females and eggs are exploited at remote beaches. Reducing the survival rate of the early life stages and thus reducing potential recruitment to the Aru feeding grounds increases the proportion of the Aru stock subject to harvesting at the Aru feeding grounds. Such a scenario predicts the extinction of the Aru stock within one generation.

Management

An incomplete understanding of local population dynamics as a result of the green turtle's complex life-history generally hampers management decision-making. For example the long-distance migration between nesting and feeding habitat and the protracted period of up to 40 years till maturity delays detection of local population declines. Furthermore, the mixed composition of a foraging aggregation (Lahanas *et al.* 1998; Dethmers *et al.* in prep. b) can result in population declines across a broader geographic area than is immediately and locally apparent. Also, temporal demographic data on population structure and abundance are scarce or non-existent in many remote regions. It is often in these remote areas where indigenous

coastal communities rely on the resource for subsistence, and where large-scale harvesting may need to be controlled to prevent extinction. We initially focused on management scenarios implemented locally that require little administrative regulations and therefore low costs, yet found that they reduce extinction risk effectively. Such scenarios are most likely to become implemented and continued. Our model showed that clutch protection on Enu Island reduced the extinction risk of the Aru stock to 50% within 50 years. However, after 200 years of clutch protection but otherwise continued exploitation, extinction risk is 100%. If, in addition to full clutch protection, adult nesting female mortality is reduced to 20%, extinction risk can be maintained at below 100% for the next 200 years. Most effective is a scenario where, in addition to clutch protection and reducing nesting female mortality, a harvest quota at the feeding grounds is maintained at 500 large females per year. Only then can a negligible extinction risk be achieved for the next 50 and even 200 years.

Implementation of identical conservation measures in remote areas does not further affect the extinction risk over 50 or 200-years. These projections are based on the assumption that 50% of individuals from this stock remain in Aru (α) throughout their life cycle and that the foraging aggregation represents 50% of all foraging stocks in the region ($\phi = 0.50$). We tested for the influence of various proportions of and found that stock with a larger sedentary proportion (e.g. $\alpha = 0.75$) slightly reduces the probability of extinction under the full local management scenario (C). Again, inclusion of remote management (scenario D) does not further improve the extinction risk of the target stock. However, if the aggregation of individuals at the Aru feeding ground is small relative to aggregations at other feeding habitats (e.g. $\phi = 0.25$), the probability of extinction of the Aru stock (with $\alpha = 0.50$) is much reduced with local management, but again little improvement with inclusion of remote management. Clearly, management intervention is best enforced locally to minimize the probabilities of extinction, regardless of α and ϕ. For a situation such as in Aru, local management can achieve the best results for stock protection, without the need for complex international co-management schemes.

Many large marine species are overexploited (Pichler & Baker 2000; Holmes & York 2003; Jackson *et al.* 2008) as a result of commercial and traditional hunting for consumption and/or international trade. The green turtle in the Australasian region is facing similar overexploitation threats. Even for a stock that shows an increasing population trend (sGBR, Chaloupka & Limpus 2001), limited turtle harvesting could result in the stock being categorized as vulnerable under IUCN criteria for listing of threatened species (Chaloupka & Limpus 2002). While the green turtle is indeed globally distributed, there is strong genetic divergence between Atlantic and Pacific populations (Bowen *et al.* 1992). The global

population of green turtles has thus evolved into separate units that might differ in terms of ecology, geology, or life-history traits. In other words, the genetic data suggest two Evolutionarily Significant Units (ESUs, Moritz 1994) - one in the Atlantic Ocean and the other in the Indo-Pacific. Therefore, inferences for the green turtle population status based on conditions within the Atlantic region cannot necessarily be extrapolated for the Indo-Pacific region. The genetically distinct stocks found within the ESUs are the units on which management should be focused (Moritz 1994). This study shows that the geographic scale on which to focus management depends on where the majority of a stock resides most of its life (α) and also on the relative proportion of that stock at its residential feeding ground (β).

As pointed out by Bowen *et al.* (2007) harvesting of turtles on a feeding ground is likely to impact a range of remote stocks. At least three distinct genetic stocks are represented at the Aru feeding grounds (Dethmers *et al.* in prep. b, see also Figure 5.1). Approximately 22% (range 14.5-30.0) of the Aru feeding aggregation is comprised of the Papua New Guinea (PNG) stock, and 10% (range 5-14.5) of the northern GBR. As illustrated here, the impact of the Aru-harvests on these stocks depends on the contributing proportion of the PNG and nGBR nesting population sizes. As in the Aru case, these stocks will likely experience multiple threats in their nesting and feeding habitats. Again, the most effective management scheme in these remote areas depends on the local genetic composition and on the stock's effective population size. The impact of turtle exploitation and the associated persistence of stocks therefore need to be assessed on a case-by-case basis.

Acknowledgements

This work would not have been possible without the assistance of Dance, Jufri, Muti, Samsu and Piter on Enu Island and at the Aru feeding grounds. KSDA and LIPI provided the necessary research permits and WWF-Indonesia provided logistic and moral support during the assessment of the commercial harvest practices in Aru. We thank J. van Groenendael, H. Possingham and P. Nienhuis for useful discussions and comments on an earlier draft of this manuscript.

6.

Exploitation, management and conservation of marine turtles in Indonesia

K.E.M. Dethmers

Manucript

Abstract

Indonesia is home to six of the seven species of marine turtle but threats in the region are significant. Marine turtles play a crucial ecological role in maintaining the health and productivity of marine food chains. Conservation and management of turtles in critical nesting, foraging, and migratory habitats is therefore essential but is compromised by the remoteness of their locations. Management of the green turtles (*Chelonia mydas*) in the Aru archipelago is complicated by a complex exploitation structure. The processes that drive and control the exploitation are discussed through an assessment of the actors implicated in the exploitation and various aspects of management are investigated through an assessment of national responsibility and management options. Three out of four identified demographic groups that are implicated in turtle exploitation come from outside of Aru and each of these groups affect spatially different areas occupied by the turtles. Management responsibilities and activities should take into account the trans-boundary nature (internationally and nationally) of marine turtles and the threats. Awareness and capacity building programs are needed to inform the public and responsible agencies of possible resource depletion and management intervention scenarios.

Introduction

Green turtle populations in Indonesia are under pressure of exploitation. Documentation on concession harvests of turtle eggs in Derawan, East Kalimantan (Wicaksono 1992) and Pangumbahan, West Java (Sloan *et al.* 1994), and of animals for the turtle meat market on Bali (e.g. Groombridge & Luxmoore 1987, Schulz 1984) show that exploitation levels have long been at alarmingly high rates. For example, between 10,000 – 30,000 turtles have been landed at the harbour in Tanjung Benoa, Bali annually since 1969 (Figure 6.1). Population declines are apparent from compilations of data sets on egg harvests in Pangumbahan (Limpus 1997) and Derawan (Wicaksono 1992), track and clutch counts in Sukamade, East Java (Arinal 1997) and Aru (Dethmers *et al.* in prep. a). Three of these sites were included in genetic analysis and all three were identified as distinct breeding stocks, or Management Units (MUs: West Java, Berau, and Aru; Dethmers *et al.* 2006). Genetic differentiation among neighbouring nesting populations is a result of long term demographic separation and maintained by strong natal homing and limited female migration between such sites. Population declines in distinct breeding stocks will therefore unlikely be compensated for by recruitment from nearby rookeries. While the tag-return data from eastern Australia show that individual turtles move between rookeries as far as 250 km apart (Limpus *et al.* 2003), the genetic analyses show that a 500 km range typically provides a more accurate picture of the scale at which female movements occur and provides a guideline for conservation planning processes (Dethmers *et al.* 2006). However, individual turtles from each of the MUs cover a much greater geographic area during development and migrations between nesting and foraging locations (Dethmers *et al.* in prep. b). As these migrations often cross district, or even, international borders the importance of joined management cannot be over-emphasized.

Delineation of management areas for each MU relies on a combination of tag returns, satellite tracking and genetic analysis of foraging and harvested populations. Tremendous progress has been made over the past decade in compiling such information, and has thus far shown links among nesting and feeding habitat in Australia, Aru, West Papua, Palau, Berau, Malaysia and the Philippines. An analysis of the foraging population in Aru and several other foraging populations in northern Australia showed that stocks migrate to distant feeding grounds to form aggregations of mixed stocks (Dethmers *et al.* in prep. b). Therefore, the impact of exploitation in one area will reach well beyond the immediate targeted stock to affect populations resident in distant areas. While information on population abundance and trends

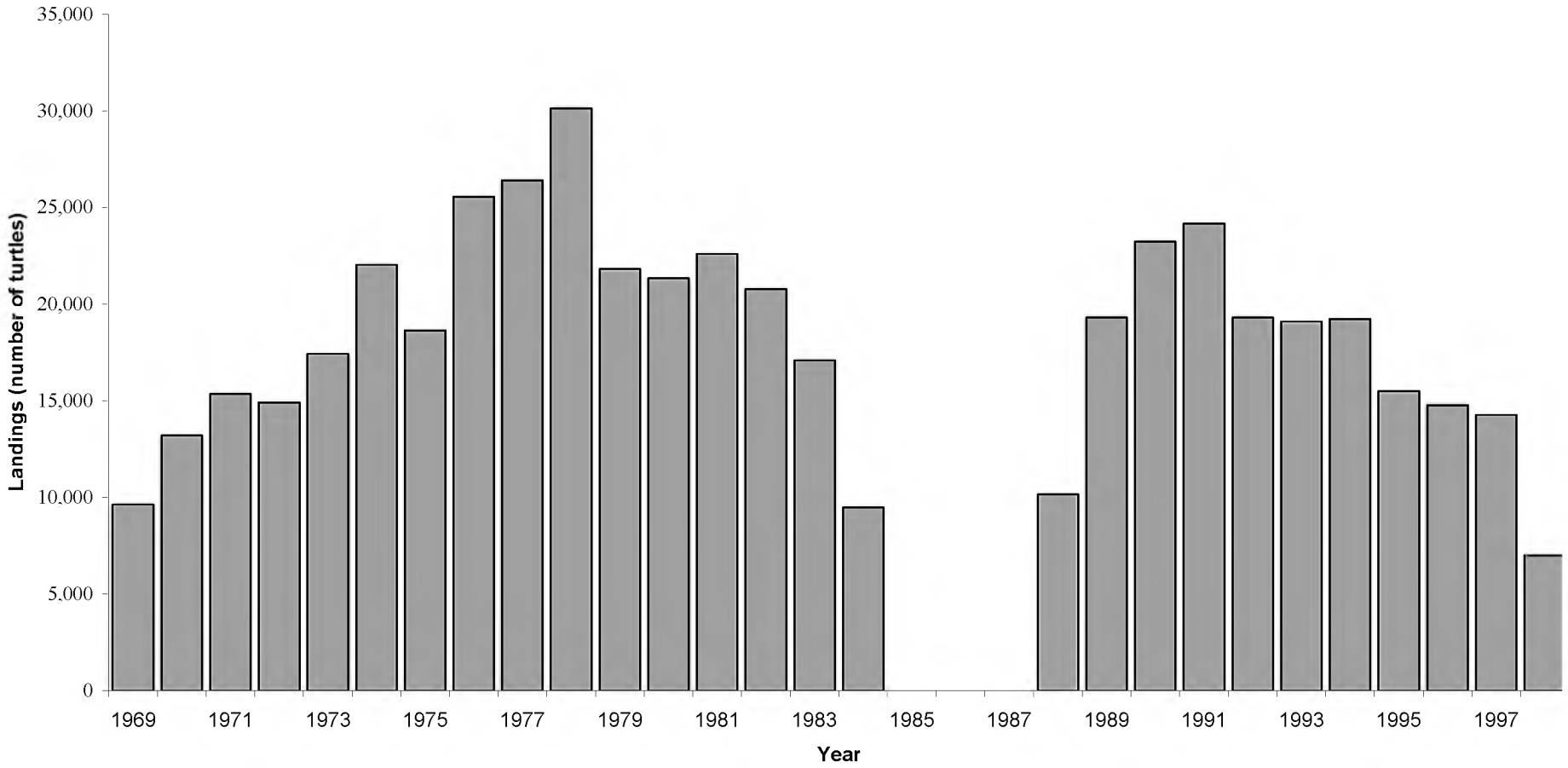


Figure 6.1. Annual number of turtles landed at the Tanjung Benoa harbour in Bali across 30 years. Data were obtained from various unpublished WWF and reports and from Schulz 1984.

in many of the other Indonesian green turtle nesting area is scattered and scarce or non-existent, inferences for these populations can be made based on the results from the genetic studies presented by Dethmers *et al.* 2006 and in prep. b). Here, I elaborate on the geographic extent of impact of green turtle exploitation in Indonesia and discuss the players involved. With a focus on the exploitation of turtles in the Aru Archipelago, I discuss the various aspects of management.

Who is responsible?

Understanding the geographic scale at which nesting areas are either demographically connected or independent is fundamental to management and monitoring of the populations that use these areas for nesting (Moritz 1994; Bowen & Avise 1996). All three of the Indonesian nesting populations that have been assessed for demographic independence (Pangumbahan, Sangalaki and Aru; Dethmers *et al.* 2006) fall within the country's national jurisdictions. In other words, strong natal homing to each of these rookeries has preserved strong genetic differentiation relative to neighbouring rookeries within and across international borders. It is therefore the responsibility of the Indonesian government to prevent population extirpation of these stocks due to reduced fecundity through egg-harvests or the take of adult females from the nesting beaches. An interesting case is the population of turtles nesting at Sanglaki and other islands in the Berau region, (Figure 6.2) East Kalimantan. Geographically, Sangalaki is separated by approximately 220 km from the Sipidan rookery in SE Sabah, Malaysia. Yet, pairwise comparisons of haplotype frequencies among nesting turtles in these two regions showed significant genetic differentiation (F_{ST} = 0.098, Dethmers *et al.* 2006). In a repeated analysis, increasing the original 29 Berau samples with an additional 38 rookery samples from the Berau rookeries analysed in a recent study (Mahardika *et al.* 2007 – unpublished report), the Berau haplotype frequencies were compared with those at all other previously analysed Australasian rookeries. The Berau and SE Sabah rookeries remained genetically distinct (F_{ST} = 0.052), thus confirming the results discussed in Dethmers *et al.* (2006). Each of these two nesting areas fall within their separate country's jurisdictions.

As most aggregations of foraging green turtles have been shown to represent several genetically distinct breeding populations (Dethmers *et al.* in prep. b), it can be expected that the population present at the Berau feeding areas calls for shared

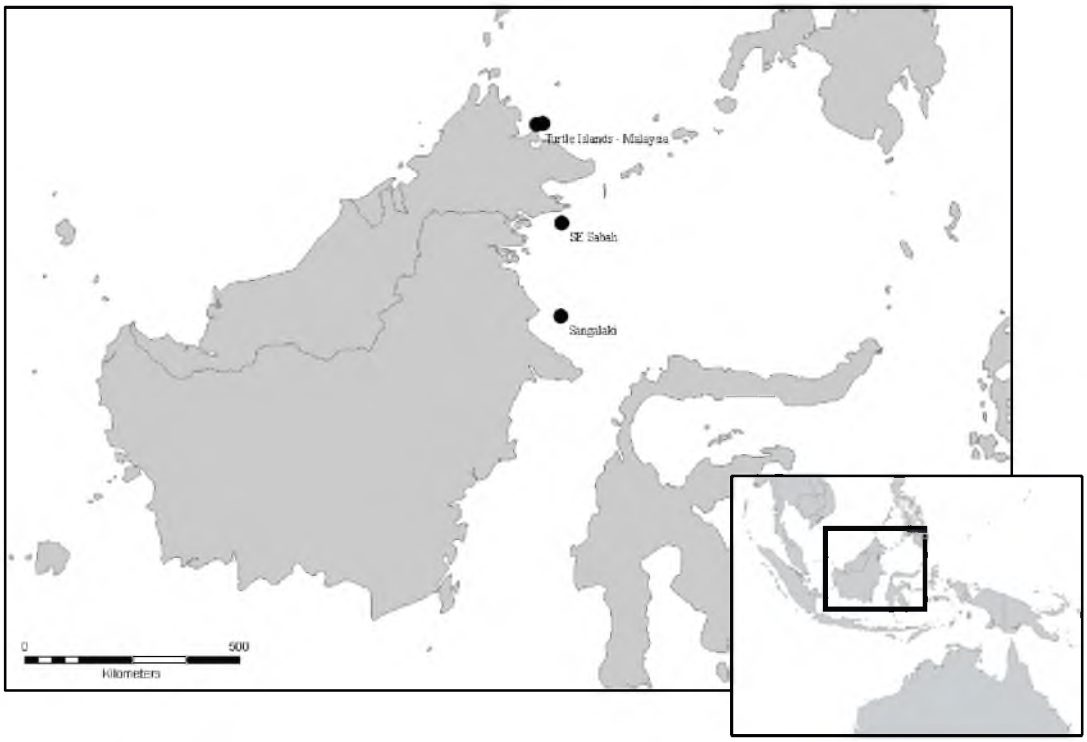


Figure 6.2. Geographic location of Sulu-Sulawesi sea rookeries

management responsibilities. The 50 samples collected and analysed at the Berau foraging aggregation by the Mahardika study were assessed using a pseudo-Bayesian maximum likelihood approach to estimate the range of likely representations of 17 genetically distinct breeding populations (referred to as stocks) to a foraging aggregation, as described in Dethmers *et al.* (in prep. for submission (b)). The analyses showed that, within a 90% confidence interval, between 19 – 41% of the individuals were representatives of Berau stock. Also the Papua New Guinea stock (5 – 18%) and the Sulu Sea stock (39 – 58%) were represented among the Berau foraging aggregation. Remarkably, turtles from the neighbouring SE Sabah stock do not appear to forage at the Berau feeding grounds, whereas the Sulu Sea stock appears to have the strongest representation. This stock has its breeding habitat at 450 km distance from the Berau feeding area. The presence of the Sulu Sea stock in the genetic analysis, confirms tag return data presented in Mahardika *et al.* (2007), where six animals, tagged while nesting at one of the Sulu Sea rookeries in Malaysia and the Philippines, were recaptured while foraging at the Berau feeding grounds.

As discussed in Dethmers *et al.* (2006), the independent breeding stocks represent distinct units for management because females are the colonisers of the nesting beaches. Management of the individual stocks is crucial to maintaining viability of meta-populations following disturbance (Avise 1995; Dethmers *et al.* in prep. c). Migratory connectivity among the stocks (as discussed in Dethmers in prep. for submission (b)) provides an indication where

responsibilities among countries or administrative regions within a country occur. The Berau case illustrates two important points; 1) strong philopatry to the rookeries of Berau and SE Sabah maintains two distinct breeding populations and individual breeding females are unlikely to shift between these rookeries. The persistence of the population of turtles nesting in Berau is therefore the responsibility of the Indonesian government; 2) a large proportion of foraging aggregation in Berau is composed of turtles from the Sulu Sea stock. Thus, at least one stock regularly migrates across the international border, indicating that Indonesia shares a responsibility with Malaysia and the Philippines to protect this Sulu Sea stock. A similar situation in Aru showed that Indonesia shares a responsibility with the neighbouring administrative district of West Papua and Palau (Dethmers *et al.* in prep. b).

Stakeholders in turtle exploitation in Aru

Various initiatives have attempted to understand the conservation status of marine turtles in Indonesia, and have formulated approaches to mitigate the major threats and provide conservation recommendations (e.g., Polunin 1975; Salm 1984; Schulz 1984; Schulz 1989; Schulz 1993; Sloan *et al.* 1994). From these studies and the observations in Aru, it is clear that an extensive, complex, active network of diverse people from throughout the archipelago was involved in the turtle trade. For example, the range of actor groups that were involved in turtle exploitation in Aru included the 1) turtle fishery operated out of Bali, 2) trawling fishery operated out of Ambon, 3) shark fishery operated out of Makassar, 4) pelagic fishery operated out of Jakarta or out of foreign countries such as Taiwan and Hong Kong, and 5) the local Arunese coastal communities. The influence of each of these groups on the exploitation was of either a direct or an indirect nature as illustrated in the actor in context diagram (Figure 6.3). For example, patrols and policing of coastal waters in protected areas or relating to endangered species is carried out by the nature conservation department (PHKA) at the sub district level but is fully dependent on financial support from the provincial office. Uncontrolled harvesting of turtles continues as long as no funding is allocated towards policing these activities. Exploitation is driven by a demand for turtle meat or products, primarily out of Bali. Through public awareness and education, conservation organisations (e.g. NGOs) aim to reduce the demand and thus reduce exploitation pressure. Although the individual contribution by each of the groups to the overall exploitation intensity was not quantified, a characterisation of each of these groups provides an insight into the complexity of the exploitation dilemma.

Trawl and pelagic fisheries

Over one hundred shrimp trawlers were active around Aru. Under US regulation regarding shrimping activities, these Indonesian trawlers have the obligatory Turtle Excluder Device (TED) on board. However, in an interview, some of the captains readily admitted that the devices generally remain unused because they greatly reduce the yield. Trawl fisheries tend to have higher discard levels than any other fishing gear type with an estimated 37.2% of total global discards resulting from trawling activities (Alverson *et al.* 1994). This is due primarily to the non-selective nature of trawl gear (Kennelly 1995). Many studies have highlighted the destructive nature of this type of fishery and its impact on sea turtles in terms of by-catch and destruction of their feeding habitat (Poiner *et al.* 1990; Nasution 1997a; Nasution 1997b). In Indonesia, this type of fishery is only allowed in the Arafura Sea and poses a serious threat to the sea turtle populations within this region. A large-scale pelagic fishery, use drift and gill nets. Gillnet and driftnet fisheries have been shown to cause substantial sea turtle mortality (Eckert 1997). The presence of these, generally foreign ships is not always appreciated. Coastal communities watch the rejected by-catch products washing up on the beaches, the resources upon which they are reliant.

Turtle fishery

While the exploitation was primarily commissioned through tradesmen in Bali, traditional Butonese and Bugis seafarers from southern Makassar do the actual catching of turtles. They arrive with large Bugis schooners (Figure 6.4), each capable of loading approximately 300 turtles. Large drift nets were set out over the seagrass beds at night, catching on average 15 turtles per night. The turtles were kept in holding pens (Figure 6.5) until a large enough number has been caught to fill the cargo (Figure 6.6). In 1998/1999, the era before the crash of the Indonesian economy, a fee of 800,000 – 1,000,000 Indonesian Rupiah (IRp, then equivalent to US$ 100-125) was paid to representatives of the coastal villages who claimed to possess the customary ownership of the exploited part of the coastal area. The money was paid in return for access to the customary exploitation zone and the construction of the holding pens. In addition to exploitation from the foraging grounds, this actor group also took turtles directly from the nesting beaches.

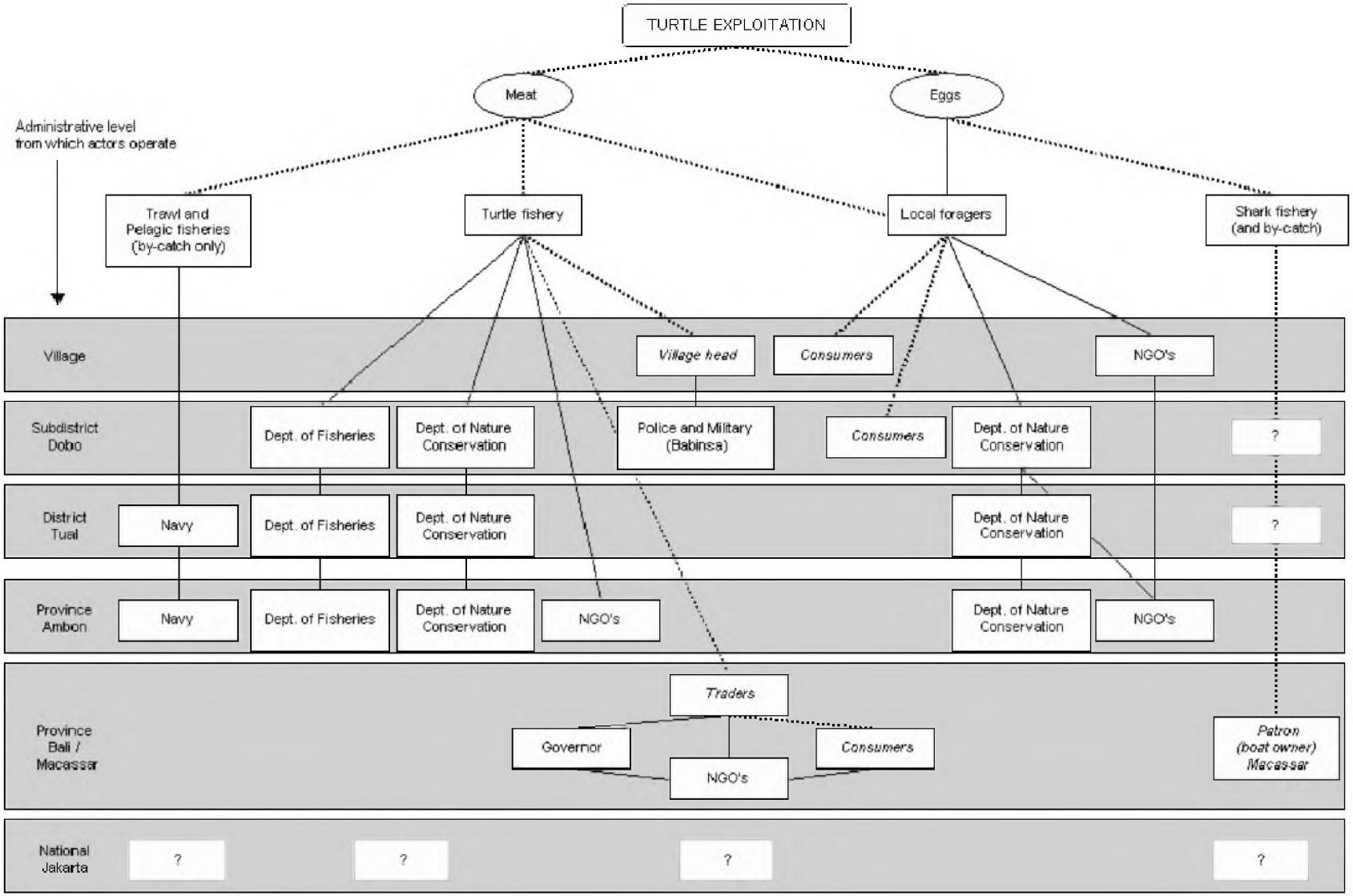


Figure 6.3. Actor in context diagram of the diverse range of consumers (in italics) that drive (dotted lines) and the responsible administrators that control (solid lines) the green turtle exploitation in Aru

Figure 6.4. Typical ship used in the Bali turtle fishery

Figure 6.6. A load of green turtles aboard a turtle vessel, ready for shipment to Bali

Figure 6.5. Holding pens were distributed across the shallow coastal waters in SE of Aru

Figure 6.7. Mixed bags of turtle meat, offal and eggs are sold from door to door

Shark fishery

This fishery, as many other fisheries in Eastern Indonesia is dominated by Bugis and Butonese fisherman from Makassar. They use baited hooks on longlines, set out throughout the Southeast Arafura Sea and even venture into Australian territorial waters. The possibility of sea turtles interacting with longline gear in Australian waters has been recognized for almost a decade (Miller 1993). Turtles are attracted either by the bait or out of curiosity and risk getting hooked or entanglement in the lines. If a sea turtle cannot reach the surface of the water to breath, its chance of drowning increases. Pelagic longline fisheries have been implicated in significant numbers of turtle deaths and as these fisheries expand the probability of sea turtle mortality from their operations increases (Gerosa & Casale 1999, Oravetz 1999, Chaloupka & Limpus 2001, Robins *et al.* 2002). During rough weather conditions, these fishermen seek shelter near Enu Island and roam the beaches in search of fresh nests. Impatient individuals have been observed to turn over the emerging female to directly extract the eggs from the ovaries, rather than await the deposition of a clutch. As the Islamic belief renders turtle meat as not pure (not halal), the remainder of the turtle is left to decompose at the beach.

Arunese coastal communities

The exploitation of turtles by members most of the communities along the East coast of Aru is primarily done on a subsistence basis, although members of certain families from Batugoyang, a village in the far Southeast, are engaged in the trade of turtle meat in Dobo (Aru's main town on the West coast). The meat, offal and eggs were sold in small bags form door to door in quantities worth Rp.1,500 (US$ 0.19, Figure 6.7). One turtle can yield up to 100 bags. Whole turtles are also sold on board of a cargo ship that visits Batugoyang once or twice a month on its way to and from West Papua.

Turtles are taken from the nesting beaches on Enu, Karang and Jeh Islands, as well as from the feeding grounds, where they are speared. The Arunese use wooden sticks with iron tip to probe the sand in search of a clutch of eggs. Motivation for exploitation varies among the communities. Some sail out specifically with the intention to catch turtles. Mostly, however, turtles are caught opportunistically within the scope of other exploitation activities, such as pearl diving, (shark) fishing, or sea cucumbers (trepang) collection. During the pearling season (which generally coincides with the southeast monsoon, see Dethmers *et al.* in prep.a), the pearl divers go ashore to collect eggs and sometimes turtles for immediate consumption aboard the pearling vessel. The Arunese shark fishery, which is small in comparison to the one operated out of Makassar as described above, primarily uses drift nets. Turtles (or other non-targeted species) that are accidentally caught are brought back to the village. While the

nets are set (usually at night) in the vicinity of any of the nesting beaches, the crew sets out to collect turtles and/or eggs at the beaches as an extension of their main activity. People from most coastal villages collect sea cucumbers (trepang) on the tidal flats at night, again, primarily during the southeast monsoon. Often they do not return to their village, but camp on the islands Jeh and Mar for a week or longer. During this period, turtle eggs and meat constitute their main diet.

Another distinction among the Arunese exploiters is based on their religious belief. Muslim villagers (approximately one-third of the total Arunese population) are not allowed to eat turtle meat and therefore will not catch turtles for private consumption but focus on egg harvests or the occasional trade of meat. People belonging to particular clans whose ancestor is or is associated with a turtle (green or hawksbill) are not supposed to collect any turtle product at all (Osseweijer 2001). Unlike inhabitants of other islands in the region, such as the Caroline islands (McCoy 1974), the Tokelau Islands (Johannes 1982), Torres Strait Islands (Johannes & MacFarlane 1991, aboriginal peoples in the Gulf of Carpentaria (Kennett *et al.* 1998), or at the nearby Kei islands (Barraud 1990; Suarez & Starbird 1996), the Arunese have no tradition or ceremonies which involve the consumption of turtle meat (Osseweijer 2001).
All Arunese people believe that their ancestors have originated from Enu and Karang islands. Therefore, the islands are sacred grounds and not supposed to be permanently inhabited. When visiting the islands, particular rules have to be observed and prior permission has to be requested from and granted by certain ancestors. Exploitation or culling of any of the terrestrial species on these islands (including invaded species such as rats) is taboo. The exploitation of turtles from the islands is exempt because it concerns a dominantly marine species.

Managing the turtle exploitation

In summary, although this research focussed primarily on the impact of the foreign turtle fishery as the main threat for the persistence of the Aru turtle population, local drivers of the turtle trade should not be disregarded. For example, a continued demand for turtle meat at both the village and the subdistrict level drives local foragers to collect turtles and their eggs (Figure 6.3) and the turtle fishery would not be able to operate if local village leaders were not to accept financial returns for the exploitation of their resources. In Indonesia, the Directorate of Nature Conservation (PHKA) is the government agency responsible for managing natural resources. With the implementation of the Aru Tenggara Marine Reserve (ATMR, see chapter 2), the department of species protection and nature conservation (BKSDA) in Ambon was

responsible for the management of all resources within the boundaries of the reserve. However, sea turtles, as an exploitable resource, used to fall under the responsibility of the department of fisheries. Thus, one governmental department managed turtles within the boundaries of the reserve, whereas another managed those that ventured outside the borders. A lack of inter-departmental communication regarding this issue, the turtle being a migratory species, and undefined delineation of the reserve long failed to protect the turtle population in Aru. When green and hawksbill turtles became listed as protected species under by Government Regulations 7/1999 and 8/1999, they became the responsibility of the PHKA. Despite this shift, the subdistrict office of fisheries in Dobo maintained control over the turtle fishery (Figure 6.2). A clear role for local and international NGOs is to inform and assist local and regional governments in their effort to curb the exploitation and prevent possible local extinctions.

There is clearly a wide range of other players active in the exploitation scenario. Detailed dynamic modelling of the impact of the individual and combined threats could reveal if indeed the turtle fishery is the main cause of the decline in the Aru population. Two general conclusions can be drawn from the above description of the exploitation. First, the severe weather conditions during the northwest monsoon, from late December to late March (see Dethmers *et al.* in prep. a), clearly prevent many of the exploitation activities to occur. This is of particular significance for the annual fecundity of the nesting population; i) the females are not disturbed during oviposition, ii) the rainfall associated with the northwest monsoon improves the substrate conditions (Dethmers *et al.* in prep. a), and iii) the clutches are left alone to incubate and hatch. I suggest that this temporal gap in the exploitation pressure on the population has so far kept the Aru stock from complete extirpation. Secondly, three of the four actor groups are primarily people from outside of Aru and each of these groups appear to affect different spatial areas occupied by the turtles; i) the pelagic fishery affects primarily the migrating individuals in the open water, ii) the turtle fishery affects the foraging turtles present at the seagrass beds, and iii) members of the shark fishery and local Arunese harvest eggs at the rookeries. This provides a temporal, as well as a spatial focus in the design of a management plan.

The complexity of implementation of co-management principles in marine resource management has been widely studied (Pomeroy & Carlos 1997, Pomeroy & Berkes 1997, Pet-Soede 2000, Harkes & Novaczek 2002, Beger *et al.* 2004, Verheij *et al.* 2004). Co-management or community-based management of turtles in Aru has great potential. As shown by Dethmers and Baxter (in prep. c) management intervention scenarios, implemented locally, are highly successful in preventing the extirpation of the Aru stock. Limiting the take of adult

females from the nesting beaches and protection of the clutches require little administrative regulations and therefore low costs, yet were found to reduce extinction risk effectively. Such scenarios are most likely to become implemented and continued. The main problem lies in the fact that there is generally very little perception of the need to protect and thus manage sea turtles (see e.g. Osseweijer 2001). Awareness and education programs that highlight the prospect of resource depletion and the options of sustainable resource use should therefore be the primary goal in establishing a full-scheme conservation program.

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Summary

Sea turtles are classified in the order of Testudines and are subdivided into two families, Cheloniidae and Dermochelyidae and seven species. On a global scale, sea turtles have undergone a considerable reduction in their abundance since pre-colonial times and six of the seven species, are listed as endangered in the IUCN red list. The green turtle (*Chelonia mydas* (Linnaeus, 1758)) has a circumglobal distribution and is known to nest in more than 80 countries world-wide. A re-assessment of the status of *C. mydas* indicated extensive subpopulation declines in all major ocean basins. Conservation successes in both the Atlantic Ocean (e.g. in Costa Rica, Florida and Ascension Island) and the Pacific Ocean (e.g. in Australia, Japan, and Malaysia) have resulted in increased abundances for some populations. However, human activities continue to pose a serious threat to the status of most populations in the Indo-Pacific region (e.g. in Vietnam, Thailand and parts of Indonesia). For the majority of the sites throughout the Indo-Pacific region, a paucity of data impedes accurate estimation of the affected populations' conservation status and as a result effective management intervention is difficult. The research presented in my thesis intended to decreasing this gap in knowledge by 1) estimating population size and characterizing reproductive success of a major *C. mydas* population 2) determining genetic relatedness of populations throughout the Indo-Pacific region 3) identification of migratory links between geographically disjoint areas and 4) estimating the probability of extinction under exploitation scenarios. For a case-study, I selected a green turtle population nesting and foraging in the Aru archipelago in far SE Indonesia because 1) Aru is believed to represent one of the remaining large green turtle rookeries and foraging aggregations in SE Asia, 2) this Aru population is subject to heavy exploitation pressure, both for local subsistence as well as for the commercial market, 3) geographically, the Aru archipelago is located in the heart of the Australasian region, and 4) some historical data on population nesting densities and exploitations rates are available.

The status of sea turtle populations is best revealed by long-term trends in population abundance. The best available measure for population abundance is counts of nesting females or nests because females repeatedly return to the same beach to nest and because this is the time in their life-cycle that turtles are accessible for direct counting (Chapter 2). Among approximately one hundred known *C. mydas* nesting areas in Indonesia, one of the largest nesting populations occurs on Enu Island in the far southeast of the Aru archipelago. During two four-months census periods in the 1997/1998 nesting season, 564 adult female turtles received an individual tag while on the beach to nest. Nesting occurs year-round, with a distinct peak between November and March. Based on the combined nest- and track-counts the total population was estimated at around 600 individuals for that year. Comparison of

nest-counts across four consecutive nesting seasons (between 1997 and 2001) showed strong fluctuations in annual nesting densities, conform the characteristic pattern in *C. mydas* nesting world-wide. Nest-counts recorded over the preceding two decades suggest that nesting densities were much higher in those years, therefore, the current data indicate a significant decline in the total population size over a short period of time (less than half a generation). Generally, morphometric observations on this population are comparable to north Australian *C. mydas* populations; mean curved carapace length of 103.6 (± 5.1) cm, mean clutch-size of 106 (± 22.5), mean incubation time of 58.6 (± 5.3) days and mean hatching success of 85.2% (± 12.8). The year 1997 was a severe El Niño year during which the nesting substrate was extremely dry, hampering the turtles in digging their nests. Only 20% of all nesting attempts in the 1997/1998 season was successful, with an observed 1.4 clutches per female. Based on a mean re-nesting interval of 5.5 years, as observed in the northern Great Barrier Reef (nGBR) nesting populations in eastern Australia, the total population of adult females nesting at Aru is estimated between 3,000 and 6,000.

While an understanding the complex life-history of this migratory species is largely obtained from long-term mark-recapture studies of females tagged while nesting on the beaches, genetic assays provide a particularly useful method for examining difficult to measure components of sea turtle life history and evolution, such as e.g. the global grouping of *C. mydas* into two assemblages corresponding to the Atlantic Ocean and the Indo-Pacific ocean basins. In my research I have used molecular analysis to examine if mature nesting female turtles return to their natal beach to deposit eggs, to establish the geographic scale of individual breeding populations and to establish how various *C. mydas* populations distributed throughout Australasia (Chapter 3). The outcomes are crucial to understanding how green turtles respond to changes in the availability of nesting sites over time and to determining the correct spatial scale for management and monitoring. A multi-scale assessment of mitochondrial DNA (mtDNA) variation among 714 samples from Aru and an additional 26 green turtle rookeries throughout the Australasian region revealed 25 haplotypes, and their frequency distributions indicated 17 genetically distinct breeding stocks (Management Units; MUs). These breeding stocks consist either of individual rookeries (e.g. Aru) or groups of rookeries that are generally separated by more than 500 kilometres (e.g. the nGBR). A unique haplotype (C14) dominates the genetic structure of the Aru population and pairwise comparisons to other nesting population in the region identified this population as a distinct MU. The effective genetic isolation of the Aru population confirms that adult female turtles return to their natal rookery. Phylogenetic analysis of the 25 haplotypes shows five clades, and reveals further partitioning of sequence divergence ($\Phi = 68.4$) between Pacific Ocean and SE Asian/Indian Ocean rookeries. This genetic distinction is the result of a land barrier

between northeast Australia and New Guinea (currently the Torres Strait) during the Pleistocene. Surprisingly, despite recurrent opening of this barrier approximately every 100,000 yrs and the proximity of the large nGBR population, genetic divergence has not been substantially eroded. Clearly natal philopatry at a subregional scale has provided an effective buffer against complete homogenisation of these regions over long time scales.

The genetic data demonstrate that gene flow among green turtle populations is likely to occur among proximate rookeries located within 500 km but only rarely among more distant ones. Significant correlation between genetic divergence and geographic distance between rookeries is apparent at spatial scales up to 2000 km (Isolation-by-distance). However, the correlation is weak compared to other species with similar dispersal capacities. Overall, the dynamic stock structure of the Australasian *C. mydas* populations is influenced by the capacity of females to relocate among proximal breeding sites. The population structure inferred from mtDNA is consistent with the scale of movements observed in long-term mark-recapture studies of east Australian rookeries.

C. mydas is a classic example of a migratory species, with adult breeding migrations between feeding and nesting habitat sometimes encompassing thousands of kilometres. The geographic extent and direction of oceanic migration varies among populations and among individuals within a population. Generally, foraging aggregations are composed of several genetically distinct source populations. The coastal seagrass habitats in the East of Aru support a large population of resident *C. mydas*. Recaptures of adult foraging turtles, tagged while nesting in the Sulu Sea region, Western Australia and the nGBR, show that individuals from these regions migrate to the Aru feeding grounds (Chapter 4). A systematic analysis of the composition of the Aru foraging aggregation reveals which regional populations reside at these feeding grounds and might be affected by the local harvesting activities. Mixed stock analysis, using mtDNA haplotypes identified among the nesting populations, studies the migratory connectivity among seven feeding grounds ($n = 463$, $F_{ST} = 0.077$) located along a 5000-kilometer transect across the north Australian coast, and the 17 genetically distinct stocks from the Indo-Pacific region. A mixed stock analysis of multiple green turtle foraging aggregations in the Indo-Pacific suggests a complex network of connectivity among nesting and feeding habitats in this region. Most sampled feeding grounds support multiple stocks. The Aru and the south-western Gulf of Carpentaria feeding grounds are dominated by a single local genetic stock; $49 \pm 8.8\%$ and $93 \pm 2.6\%$ percent representation respectively. Geographic distance between breeding and feeding habitat strongly influences representation of a stock at a feeding ground ($w_i = 0.654$) but is not a good predictor of the extent of that representation. Besides the Aru stock, the Papua and nGBR stocks are also represented at the Aru feeding grounds. None of the other assessed feeding grounds supported individuals from the Aru

breeding stock, suggesting that turtles nesting on Aru do not migrate south into Australian waters. However, relatively large standard errors around the estimated contributions make robust inferences difficult. This is in part due to reduced analytical power associated with shared common haplotypes, but also because incomplete sampling of nesting populations in some areas may have biased the results, particularly by reducing the capacity to detect contributions from more distant rookeries. The differential proportional contributions demonstrate that impact of local exploitation on the persistence of remote nesting populations should be assessed on a case-by-case basis.

C. mydas is exploited for its meat and calipee to trade or for subsistence. Harvests and net entanglements are the major sources of anthropogenic mortality in the Indo-Pacific region, causing rapid local and regional declines. In Aru, *C. mydas* became heavily exploited on a commercial basis, starting in the early 1980s. Exploitation occurs both at the nesting beaches and at the foraging grounds. Because multiple, independently breeding stocks reside at the foraging grounds, the impact of the local exploitation has a geographically broad impact. Thus, while globally *C. mydas* may persist as a result of local conservation successes, regional populations are threatened with extirpation. The viability of the Aru breeding stock and that of remote stocks migrating to the Aru feeding grounds was investigated using demographic and genetic data in an age-based model (Chapter 5). Based on best available data, assembled locally (see chapters 2, 3, and 4) and extrapolated from the nGBR stock, this model provides the most reliable approach to determining stock persistence. In this chapter the 'Aru stock' refers to all individuals that return to Enu Island each breeding cycle, and the 'Aru population' refers to all individuals that can be observed within the Aru archipelago throughout most of their life cycle. The model predicts that both the Aru stock and the Aru population can persist under a local harvest of 3,000 adult individuals per year in the absence of other anthropogenic or natural sources of mortality. However, the composition of both the Aru stock and population is expected to change: the proportion of long-distance migrants increases relative to individuals that forage and nest in Aru. Increasing exploitation levels increase the probability of extinction. The worst scenario considered includes exploitation of turtles at all feeding grounds in the region, as well as exploitation of adult females and eggs at all nesting areas linked to Aru. The model predicts that the Aru stock will be extinct within 50 years under this scenario. I have used this scenario to assess several possible management strategies that might reduce extinction risk for the Aru stock. Protection of the nests at the Enu rookery has little effect if this were the only type of management intervention. If in addition female mortality at Enu is reduced to zero, the risk of extinction is expected to be 50% after 200 years. Management intervention that includes a maximum quota at the Aru feeding grounds of 500 adult turtles per year maintains low probabilities of extinction. This number

would suffice to supply for the local subsistence market. Incorporation of similar management scenarios at remote nesting and foraging grounds does not further improve persistence of the Aru stock.

Green turtle populations in Indonesia are under pressure of commercial and subsistence exploitation. To determine best-practise and cost-efficient management scenarios as well as governing responsibilities, each case needs to be assessed separately, while considering the cross-boundary nature (internationally and nationally) of marine turtle life-histories and threats (Chapter 6). Various forms of exploitation affect the green turtle population in Aru and the collective impact it poses on the persistence of this population requires adequate management and monitoring intervention. The combined results described in the four data chapters of this thesis, show that this management intervention is best enforced locally in the Aru archipelago to minimize the probabilities of extinction, without the immediate need for complex international co-management schemes. This study underpins that, on a regional scale, conservation of *C. mydas* must be informed by a detailed understanding of the spatial distribution of stocks, their inter-relatedness, and local population dynamics. Conservation management strategies as argued in chapter 6, should reflect this understanding, but also acknowledge the social concerns that accompany biodiversity conservation.

Future research

There are still uncertainties that complicate an accurate projection of the green turtle population in Aru and other parts of Indonesia. For example, without a reliable estimate of the size of a feeding aggregation, proportions of different contributing stocks at a feeding ground cannot be translated into real numbers of individuals (the 1- ß in the population viability analysis discussed in Chapter 5). It therefore remains unclear whether exploitation at a feeding ground will a have severe impact on remote stocks. Furthermore, a stock can sustain certain levels of exploitation without facing extirpation depending on the size and migration behaviour of any stock (Chapter 5). Future studies should therefore focus on obtaining this type of information, for example through genetic analysis that focus on identifying differentiation among turtles that migrate to remote nesting beaches and those that remain on the Aru feeding grounds.

Population declines have been linked to change of conditions in foraging habitat, nesting beaches, and migratory areas. Early warning systems of population change may become apparent in alterations to breeding rates, growth rates, sex ratios of young recruits, or a changing demographic within foraging areas. The information presented in this thesis, supplemented with datasets from other studies form a strong baseline for a population

viability analysis (PVA) framework that would couple spatial (use of the landscape), demographic, and ecosystem processes (ecological interaction) to best forecast human impacts (e.g. exploitation). Thus, continued collection of baseline and experimental data across species and populations dealing with reproductive cycles, physiological control systems, and pertinent ecological parameters is of paramount importance. Otherwise, in times of rapidly changing environments, we will not have the necessary information to assess possible and probably impacts on sea turtle populations and apply early and appropriate management practices.

Samenvatting

De zeven bestaande soorten zeeschildpadden zijn geclassificeerd in the orde van de Testudines welke bestaat uit twee families (de Cheloniidae en de Dermochelyidae). Sinds de opkomst van de koloniale handel, is het aantal zeeschildpadden wereldwijd sterk afgenomen, met als gevolg dat zes van de zeven soorten op de rode lijst voor beschermde soorten van de IUCN staan. De soepschildpad (*Chelonia mydas* (Linnaeus, 1758)) heeft een wereldwijde verspreiding en heeft haar nestgebied in meer dan 80 landen. In een herwaardering van de *C. mydas* stand, werd een sterke achteruitgang vastgesteld van subpopulaties in alle oceaangebieden. Succesvolle beschermingsprogramma's in zowel de Atlantische Oceaan (Costa Rica, Florida en Ascension Island) en de Stille Oceaan (Australië, Japan, en Maleisië) hebben weliswaar geleid tot een toename in sommige populaties die hier voorkomen, maar menselijke activiteiten vormen nog altijd een grote bedreiging voor de stand van de meeste populaties in de Indo-Pacifische regio (zoals in Vietnam, Thailand en Indonesië). Een gebrek aan demografische data van deze soort in het merendeel van de Indo-Pacifische regio belemmert een betrouwbare inschatting van het effect van de antropogene activiteiten op getroffen populaties. Dit heeft tot gevolg dat ontwikkeling en invoering van gerichte beheersmaatregelen moeizaam is. Het doel van mijn onderzoek, beschreven in dit proefschrift, is dan ook een invulling te geven aan deze kennisklooof door: 1) de verspreiding van en relaties tussen *C. mydas* populaties in de Australazische regio in kaart te brengen, 2) de populatie dynamiek en het voortplantingssucces van een belangrijke *C. mydas* populatie te analyseren (geselecteerd als case-study), en 3) een inschatting te maken van de kans op uitsterven als gevolg van exploitatiedruk. De soepschildpaddenpopulatie in de Aru archipel, in het verre zuidoosten van Indonesië, diende als een case-study omdat: 1) voorgaande studies suggereren dat Aru het nest- en fourageergebied is van één van laatst overgebleven, grote populaties in Zuidoost Azië, 2) deze Aru populatie onder grote exploitatiedruk staat van zowel lokale vangsten voor levensonderhoud als van commerciële vangsten, 3) de Aru archipel gelegen is in het geografische hart van de Australazische regio, en 4) er historische gegevens bekend zijn van deze populatie waardoor eventuele trends kunnen worden bestudeerd.

De stand van zeeschildpaddenpopulaties kan het best in kaart worden gebracht door veranderingen in lange termijn waarnemingen van populatiedichtheden. Dergelijke waarnemingen in zeeschildpaddenonderzoek worden doorgaans gedaan door het tellen van eierleggende vrouwtjes, aangezien zij regelmatig terug keren naar hetzelfde strand om te nestelen en omdat dit het moment is in de levenscyclus van een zeeschildpad waarop zij het best te observeren en te tellen valt (Hoofdstuk 2). Van de ongeveer honderd bekende

leggebieden voor *C. mydas* in Indonesië, heeft het strand op Enu, een eiland in het zuidoosten van de Aru archipel, een van grootste legpopulaties van zuidoost Azië. Gedurende twee censusperioden van elk vier maanden in het legseizoen van 1997/1998, werden 564 volwassen vrouwtjes voorzien van een uniek merk nadat ze het leggen hadden volbracht. Nestelen gebeurt gedurende het hele jaar, met een duidelijke piek in de periode tussen november en maart. Op basis van de gemerkte vrouwtjes en het aantal waargenomen nesten, werd de nestelende cohort voor dat seizoen geschat op 600 individuen. Het aantal nesten fluctueerde sterk over de vier achtereenvolgende jaren 1997-2001, wat in overeenstemming is met het wereldwijde, karakteristieke legpatroon van *C. mydas*. Echter, de tellingen waren beduidend lager dan die van de voorgaande twee decennia, hetgeen suggereert dat de nestelende populatiedichtheid is afgenomen en wel in een relatief korte periode (minder dan een halve generatie).

Morfometrische waarnemingen van deze populatie zijn vergelijkbaar met noord Australische *C. mydas* populaties, zoals de gemiddelde gebogen carapax lengte van 103.6 (± 5.1) cm, een gemiddelde broedgrootte van 106 (± 22.5) eieren, een gemiddelde incubatie tijd van 58.6 (± 5.3) dagen, en een gemiddeld uitkomst succes van 85.2% (± 12.8). Het jaar 1997 was een zwaar El Niño jaar waarin extreme droogte en hoge temperaturen ertoe bijdroegen dat het nest substraat extreem rul was, waardoor de schildpadden grote moeite hadden een nest te graven. Slechts 20% van alle legpogingen in het seizoen van 1997-98 waren succesvol, met een waargenomen gemiddelde van 1.4 broedsels per vrouwtje. Bij een aanname van gemiddeld 5.5 jaar tussen twee legseizoenen, zoals waargenomen bij nest populaties in de noordelijke Great Barrier Reef (nGBR) in oost Australië, wordt de totale populatie van volwassen vrouwtjes geschat tussen de 3.000 and 6.000.

Lange termijn vangst-terugvangst studies van gemerkte volwassen vrouwtjes hebben in belangrijke mate bijgedragen tot een inzicht in de complexe 'life-history' van deze migrerende soort. Dankzij genetische methodieken is het nu ook mogelijk om moeilijk te bestuderen componenten van de zeeschildpadden zoals de levenscyclus en evolutie nader te onderzoeken. Zo is er bijvoorbeeld een significant genetische splitsing waargenomen tussen *C. mydas* populaties in de Atlantische Oceaan en de Middellandse Zee, en populaties in de Indische en Stille Oceaan. In mijn onderzoek heb ik gebruik gemaakt van moleculaire analyses om aan te tonen dat volwassen, nestelende vrouwtjes terugkeren naar het strand waar ze uit het ei zijn gekropen (het geboortestrand) om daar vervolgens zelf eieren te leggen. Met behulp van deze techniek heb ik ook de geografische schaal vastgesteld op basis waarvan niet alleen zich individueel voortplantende populaties kunnen worden onderscheiden maar ook de verspreiding van genetisch verschillende populaties binnen Australazië (Hoofdstuk 3). De

resultaten zijn cruciaal om te begrijpen hoe de soepschildpad reageert op veranderingen in de beschikbaarheid van nestgebieden en tevens om de juiste ruimtelijke schaal voor beheer en monitoring vast te kunnen stellen.

In totaal werden 714 huidmonsters van de nestelende Aru schildpadden en van individuen van 26 andere Australazische populaties geanalyseerd voor mitochondriaal DNA (mtDNA). Er werden 25 verschillende haplotypen gevonden en door middel van een analyse van de frequentieverdeling konden 17 genetisch verschillende subgroepen worden geïdentificeerd. Omdat deze subgroepen zich niet onderling voortplanten, worden de afzonderlijke subgroepen gezien als onafhankelijke beheerseenheden (Management Units; MUs). De subgroepen bestaan uit schildpadden uit ofwel een enkel nestgebied (b.v. Aru) of uit een groep van nestgebieden die gemiddeld niet verder dan 500 kilometer van elkaar verwijderd zijn (b.v. de nGBR). De genetische structuur van de Aru populatie wordt gedomineerd door een uniek haplotype, C14, en paarsgewijze vergelijkingen met andere nestpopulaties in de regio geeft aan dat deze Aru populatie een onafhankelijke beheerseenheid is. De effectieve genetische isolatie van de Aru populatie bevestigt dat de vrouwtjes terugkeren naar hun geboortestrand. Door middel van fylogenetische analyse van de 25 haplotypen kunnen vijf clades worden vastgesteld. Bovendien is er een sequentie-divergentie (Φ = 68.4) aan te tonen tussen nestgebieden in de Stille Oceaan en die in de Zuidoost-Aziatische wateren en de Indische Oceaan. Deze genetische distinctie is het gevolg van een landbarrière uit het Pleistoceen tussen noordoost Australië en Nieuw Guinea (de huidige Torres Straat). Een interessante observatie is dat, ondanks herhaaldelijk openen en sluiten van de landbarrière (ongeveer elke 100.000 jaar) en de nabijheid van de omvangrijke nGBR populatie, de genetische divergentie niet aanzienlijk is geërodeerd. Klaarblijkelijk heeft natale philopatrie op een subregionale schaal voor een effectieve buffer tegen volledige homogenisatie binnen deze gebieden gezorgd.

De genetisch data tonen aan dat genmigratie zich voordoet tussen soepschildpad populaties op 500 km afstand van elkaar, maar dat dit slechts zelden gebeurd over grotere afstanden. Er bestaat een significante correlatie tussen genetische afwijking en geografische afstand tussen nestgebieden op hoogstens 2000 km afstand ('isolation-by-distance'). Echter, de waargenomen correlatie is zwak in vergelijking met andere soorten die een vergelijkbare dispersie capaciteit hebben. De dynamische structuur van de Australazische *C. mydas* subgroepen wordt beïnvloed door de capaciteit van vrouwtjes om zich te verplaatsen naar nabijgelegen nestgebieden. De populatie structuur, zoals afgeleid uit de mtDNA analyses, komt overeen met de schaal waarbinnen verplaatsing is waargenomen binnen de lange termijn vangst-terugvangst studies van oost Australische populaties.

C. mydas is een klassiek voorbeeld van een migrerende soort. Volwassen dieren migreren heen en weer tussen nest- en foerageer habitat, soms wel over afstanden van meer dan 1000 kilometer. De afstand en koers van de migratieroute verschilt tussen populaties en tussen individuen binnen een populatie. Fouragerende populaties bestaan over het algemeen uit meerdere genetisch afwijkende bronpopulaties. Een grote *C. mydas* populatie is gevestigd bij de zeegrasvelden langs de kust van oost Aru. Terugvangsten van gemerkte volwassen schildpadden uit de Sulu zee regio, west Australië, en de nGBR tonen aan dat de in Aru fouragerende schildpadden naar deze verafgelegen gebieden migreren om te nestelen (Hoofdstuk 4). Een systematische analyse van de samenstelling van de fouragerende Aru populatie toont aan welke populaties uit de regio op de zeegrasvelden van Aru gevestigd zijn en mogelijk aangetast worden door de lokale zeeschildpadden exploitatie. De migrerende connectiviteit tussen foerageer- en nestgebieden kan door 'Mixed stock' analyse worden vast gesteld.

In totaal heb ik 463 huidmonsters geanalyseerd op mtDNA, afkomstig van schildpadden van zeven verschillende foerageergebieden, gelegen op een 5000 km transect langs de noord Australische kust en Aru (totale genetische differentiatie F_{ST} = 0.077). De 'Mixed stock' analyse vergelijkt de haplotype frequentieverdeling op de foerageergebieden met dat van de reeds geanalyseerde nestgebieden in de Indo-Pacifische regio. Het resultaat is een complex netwerk van connectiviteit tussen de verschillende nest- en foerageergebieden. Elk van de foerageergebieden huisvest meerdere genetische subgroepen, maar Aru en het zuidwestelijke deel van de Golf van Carpentaria worden sterk gedomineerd door een enkele lokale genetische stam (respectievelijke vertegenwoordiging van 49% ± 8.8 and 93% ± 2.6). De afstand van een nestgebied tot een foerageergebied is sterk bepalend of een subgroep al dan niet voorkomt in een foerageergebied (w_i = 0.654), maar het zegt niets over het proportionele aantal mate waarin de stam vertegenwoordigd is in het foerageergebied. In de zeegrasvelden van Aru komen naast de Aru-subgroep, ook subgroepn van Papua Nieuw Guinea en de nGBR voor. De Aru subgroep werd op geen van de overige zes foerageergebieden waargenomen, hetgeen suggereert dat de schildpadden die op Aru nestelen niet naar Australische wateren migreren. Echter, de standaardfout rond de geschatte procentuele aantallen in de foerageergebieden was vrij groot, zodat de resultaten niet voor verregaande conclusies bruikbaar zijn. De grote standaardfout wordt deels veroorzaakt door beperkingen in de analyse als gevolg van enkele veelvoorkomende en wijdverspreide haplotypen. Een tweede oorzaak is gelegen in het feit dat enkele kleine, mogelijk bijdragende nestpopulaties in sommige delen van Australazië niet zijn bemonsterd, waardoor de resultaten niet 100% volledig zijn. Evenwel kan uit de verschillende proportionele bijdragen geconcludeerd worden

dat de gevolgen van lokale exploitatie activiteiten op het voortbestaan van veraf gelegen nestpopulaties per geval moet worden beoordeeld.

De soepschildpad wordt met name gevangen voor zijn vlees en 'calipee', een gelatineachtig weefsel direct onder het plastron, dat wordt gebruikt voor de bereiding van schildpaddensoep. De grootste oorzaken van antropogene sterfte onder de Indo-Pacifische zeeschildpadden zijn de vangst en de verstrikking van schildpadden in visnetten, waardoor een sterke lokale en regionale achteruitgang in de zeeschildpaddenstand wordt geconstateerd. Sinds het begin van de jaren '80, wordt de *C. mydas* op grote schaal commercieel geëxploiteerd in Aru. De dieren worden zowel op de neststranden als in de foerageergebieden weggevangen. Aangezien een foeragerende populatie uit meerdere, genetisch onafhankelijke, stammen bestaat is het aannemelijk dat lokale exploitatie een geografisch wijd verbreid effect heeft. Dus, ofschoon op wereldwijd niveau de soepschildpadden stand lijkt te volharden dankzij effectieve beschermingsprogrammas, dreigen populaties op regionaal nivau uit te sterven.

De levensvatbaarheid van de Aru subgroep, evenals dat van naar Aru migrerende subgroepen, heb ik onderzocht door middel van een leeftijdsgebonden populatiemodel waarbij demografische en genetisch data zijn gebruikt (Hoofdstuk 5). Als gevolg van de complexe 'life-history' en late geslachtsrijpheid bij soepschildpadden zijn volledige demografische parameters van slechts weinig populaties bekend. Om het voortbestaan van de Aru subgroep zo betrwoubaar mogelijk te benaderen moest, naast de lokaal verzamelde gegevens (zie hoofdstukken 2, 3, en 4), gebruik gemaakt worden van geëxtrapoleerde gegevens van de nGBR subgroep. In hoofdstuk 5 slaat de "Aru subgroep" op alle schildpadden die ieder broedseizoen naar het eiland Enu migreren om te nestelen en de "Aru populatie" op alle schildpadden die het merendeel van hun levenscyclus in de Aru archipel doorbrengen. Het model voorspelt dat zowel de Aru stam als de Aru populatie de druk van een vangst van 3.000 volwassen individuen per jaar kan weerstaan zolang er geen druk is van andere vormen van sterfte, bijvoorbeeld als gevolg van bijvangst in de visserij, habitat destructie, of natuurlijke catastrofes. Echter, volgens het model zal de samenstelling van zowel de Aru stam als de Aru populatie veranderen: het proportionele aantal lange afstands migranten zal toenemen ten opzichte van het aantal residentiële individuen die in Aru zowel nestelen als foerageren. Bij een toenemende exploitatiedruk neemt de de kans op uitsterven toe.

In het meest sombere scenario dat is getoetst worden: a) schildpadden geoogst in alle foerageergebieden in de regio, b) volwassen vrouwtjes van de aan Aru gelinkte legstranden weggevangen en c) eieren geraapt. Binnen dit scenario voorspelt het model dat de Aru subgroep binnen 50 jaar is uitgeroeid. Ik heb dit scenario als uitgangspunt genomen om het

effect van verschillende beheersmaatregelen te toetsen die de uitstervingskans van de Aru subgroep zouden kunnen verminderen. Bescherming van de nesten op het strand van Enu heeft weinig effect als daarnaast geen andere maatregelen worden genomen. Wanneer naast totale bescherming van de eieren ook de vangst van de volwassen nestelende vrouwtje op Enu wordt gestopt, dan is er nog altijd 50% kans dat de populatie over 200 jaar is uitgestorven. Alleen wanneer ook een quotum van 500 volwassen schilpadden per jaar wordt ingesteld voor de vangst vanaf de zeegrasvelden op Aru, wordt de kans op uitsterving binnen 200 jaar, zo goed als nihil. Dit aantal van 500 schildpadden per jaar zou voldoende zijn om de lokale markt te voorzien. Wanneer dergelijke beheersmaatregelen tegelijkertijd in de omliggende gebieden worden toegepast, zal het toekomst beeld voor de Aru subgroep niet verder verbeteren. Uiteraard zullen wel de daar verblijvende subgroepen een vergrootte handhavingskans hebben.

Soepschildpadden populaties in Indonesia staan onder exploitatiedruk van zowel lokale vangsten voor levensonderhoud als van grote commerciële vangsten. Om te bepalen wat de meest doeltreffende en kost-efficiente beheersmaatregelen zijn moet iedere situatie afzonderlijk bekeken worden (Hoofstuk 6). De populatie soepschildpadden in Aru staat bloot aan verschillende vormen van exploitatie die tezamen een serieuse bedreiging vormen voor het voortbestaan van deze populatie. De gezamelijk resultaten, beschreven in de hoofdstukken 2 t/m 5 van dit proefschrift geven aan dat lokaal toegepaste beheersmaatregelen voor de Aru population heel effectief zijn om de kans op uitsterving te minimaliseren. Voor deze situatie is het dus niet direct nodig om complexe internationale samenwerkings projecten op te zetten. Dit onderzoek laat zien dat, voor de bescherming van *C. mydas* op een regionaal niveau, het noodzakelijk is een goed beeld te verkrijgen van de ruimtelijke spreiding van subpopulaties, hoe zij met elkaar in verband staan en wat de lokale populatie dynamiek is. Hoofdstuk 6 bediscusieerd dat beheers strategien een dergelijk inzicht zouden moeten reflecteren, met in achtneming van de sociale belangen die gepaard gaan met biodiversiteits beheer.

Verder onderzoek

Er zijn nog altijd onzekerheden die een accurate projectie van de soepschildpadden populatie in Aru en andere delen van Indonesie bemoeilijken. Bijvoorbeeld, zonder een betrouwbare schatting van fouragerende populatie grootte kunnen de proportionele bijdragen van verschillende subgroepen niet vertaald worden naar werkelijke aantallen (de 1- ß in de populatie vatbaarheids analyse van hoofdstuk 5). Het blijft daarom onduidelijk of de exploitatie op een lokaal aanwezig deel van een verafgelegen populatie van grote invloed is op de gehele populatie uit dat gebied. Een subgroep kan een zekere exploitatie druk verdragen afhankelijk van de grootte en het migratie patroon van die subgroep (hoofdstuk 5). Verder

onderzoek zou zich moeten richten op het verkrijgen van dit soort informatie, bijvoorbeeld door middel van een mogelijke genetische differentiatie tussen schildpadden die migreren naar veraf gelegen gebieden en schildpadden die dat niet doen.

Populatie verval wordt wel in verband gebracht met een verandering in de toestand van fourageer, nest en migratie gebieden. Vroegtijdige waarschuwingen voor een verandering in de toestand van een populatie uitten zich in bijvoorbeeld gewijzigd reproductie snelheid, groei snelheid, sex ratios van jonge nieuwkomers, of een veranderde demography op fourageer gebieden. De in dit proefschrift gepresenteerde gegevens, tezamen met data van andere studies vormen een sterke basis voor een populatie haalbaarheids analyse waarin ruimtelijke (het gebruik van de het zee-landschap), demographische en ecosysteem processen (ecologische interacties) worden gekoppeld om menselijke effecten op schildpadden populaties zo nauwkeurig mogelijk te voorspellen. Het is daarom van groot belang om baseline en experimentele data te blijven verzamelen over reproductie cycli, fysiologische controle systemen, en relevante ecologisch parameters van soorten en populaties.

Anders zullen we, in tijden van een snel veranderend milieu, niet de nodige informatie hebben om mogelijke effecten zijn op schildpadden populaties in te kunnen schatten en eventueel tijdig in the grijpen.

Ringkasan

Penyu laut termasuk dalam orde Testudines yang terbagi ke dalam 2 famili, *Cheloniidae* dan *Dermochelyidae,* dan tujuh spesies. Secara global kelimpahan penyu laut mengalami penuruan sejak era pre-kolonial dan saat ini enam dari tujuh spesies telah dimasukan dalam daftar merah IUCN. Penyu Hijau (*Chelonia mydas* Linnaeus, 1758) memiliki distibusi global dan habitat bertelurnya diketahui tersebar di lebih dari 80 negara di dunia. Sebuah kajian ulang terhadap status populasi *C. mydas* menunjukan adanya penurunan jumlah sub-populasi di seluruh perairan di dunia. Keberhasilan upaya konservasi baik di samudra Atlantik (misalnya do Costa Rica, Florida dan Ascension Island) maupun Samudra Pasifik (misalnya di Australia, Jepang dan Malaysia) menyebabkan peningkatan kelimpahan beberapa kelompok populasi penyu hijau. Namun demikian, aktivitas manusia yang terjadi secara terus menerus menimbulkan ancaman yang sangat serius bagi populasi penyu hijau di kawasan Indo-Pasifik (seperti di Vietnam, Thailand dan sebagian perairan di Indonesia). Kelangkaan data untuk kebanyakan tempat di kawasan Indo-Pasifik menjadi kendala untuk melakukan estimasi yang akurat untuk mengetahui status konservasi dari populasi yang terancam, sehingga upaya intervensi konservasi yang efektif sulit dilakukan. Penelitian yang dipaparkan dalam disertasi ini dilakukan untuk mengisi kesenjangan pengetahuan seperti diuraikan di atas, melalui: 1) Pendugaan besaran populasi dan keberhasilan perkembang-biakan dari salah satu populasi *C. mydas*, 2) Menentukan keterkaitan antar populasi penyu hijau yang ada di seluruh kawasan Indo-Pacifik, 3) Mengidentifikasi keterkaitan migrasi antara areal yang secara geografis terputus, dan 4) Menduga kemungkinan (probabilitas) terjadinya kepunahan dengan memakai beberapa scenario tingkatan eksplotasi. Populasi penyu hijau yang bertelur dan melakukan aktivitas makan di perairan kepulauan Aru, kawasan timur Indonesia dipilih sebagai lokasi untuk studi kasus ini. Latar belakang pemilihan lokasi penelitian ini adalah 1) Kepulauan Aru dipercayai merupakan perwakilan dari salah satu tempat peneluran dan tempat makan utama bagi populasi penyu yang ada di kawasan perairan Asia Tenggara; 2) Populasi penyu hijau di perairan Aru menjadi sasaran eksplotasi yang sangat serius baik untuk tujuan subsisten maupun komersial; 3) Secara geografis, kepulauan Aru terletak di 'jantung' kawasan Australasia; dan 4) tersedianya beberapa data histories tentang kepadatan populasi penyu hijau yang bertelur dan laju pemanfaataan.

Status populasi penyu laut sangat baik bila digambarkan dalam kecenderungan kelimpahan populasi dalam jangka waktu yang panjang. Metoda yang tepatuntuk menduga kelimpahan populasi adalah jumlah individu betina yang bertelur atau jumlah sarang karena individu penyu betina bertelur berulang kali di pantai yang sama, yang merupakan bagian dari siklus hidup penyu. Pada saat musim peneluran, jumlah individu penyu betina mudah dihitung

secara langsung (Bab 2). Di antara sekitar seratus tempat peneluran *C. mydas* yang diketahui di Indonesia, Pulau Enu di bagian tenggara kepulauan Aru diketahui merupakan merupakan salah satu tempat yang memiliki populasi penyu bertelur terbesar. Pada dua sensus yang dilakukan selama empat bulan pada musim peneluran 1997/1998, 564 ekor penyu hijau betina diberi tanda pada saat naik ke pantai untuk bertelur. Aktivitas peneluran berlangsung sepanjang tahun dengan masa puncak peneluran berlangsung antara bulan November dan Maret. Dengan menggunakan metoda kombinasi penghitungan sarang dan jejak, jumlah populasi diperkirakan 600 individu bertelur dalam periode tahun dimana sensus dilakukan. Perbandingan penghitungan sarang selama empat musim peneluran secara berturut-turut (antara 1997 dan 2001) menunjukan adanya fluktuasi kepadatan aktivitas peneluruan yang jelas, sesuai dengan pola karakteristik *C. mydas* secara global. Hasil perhitungan jumlah sarang selama dua dekade sebelumnya menunjukan bahwa kepadatan aktivitas peneluran sangat tinggi. Karena itu, data yang ada sekarang ini menunjukan penurunan besaran populasi yang signifikan dalam periode waktu yang cukup pendek (kurang dari seperdua generasi). Secara umum, data morfometri populasi penyu hijau pada lokasi penelitian sebanding dengan populasi penyu hijau yang ada di Australia bagian utara; rata-rata panjang lengkung karapas adalah 103.6 (± 5.1) cm, rata-rata jumlah telur dalam satu sarang adalah 106 (± 22.5), rata-rata masa inkubasi adalah 58.6 (± 5.3) hari dan rata-rata kesuksesan penetasan adalah 85.2% (± 12.8). Pada tahun 1997 fenomena *El Niño* terjadi yang menyebabkan kondisi substrat di pantai peneluran menjadi sangat kering sehingga mengganggu penyu pada saat proses penggalian sarang. Hanya 20% dari upaya peneluran yang berhasil membuahkan sarang yang berisi telur di dalamnya dan setiap individu betina meletakan 1.4 sarang selama musim peneluran 1997/1998. Berdasarkan data interval waktu remigrasi yang diamati pada pantai peneluran di bagian timur Australia (bagian utara *Great Barrier Reef,* nGBR) yakni 5.5 tahun, maka jumlah populasi penyu betina yang bertelur di Aru diperkirakan berjumlah antara 3000 hingga 6000 individu.

Sejauh ini studi mengenai *'mark recapture'* penyu (monitoring penyu yang ditandai) di pantai peneluran dalam waktu yang panjang dilakukan untuk memahami sejarah hidup penyu yang sangat kompleks. Namun demikian berbagai hasil studi genetik menghasilkan metode analisa untuk mengetahui beberapa komponen sejarah hidup penyu dan proses evolusi yang dialaminya, seperti pengelompokan populasi penyu hijau secara global ke dalam dua kelompok populasi besar yakni yang hidup di lautan Atlantik dan Indo-Pasifik. Dalam penelitian ini, analisa molekuler dilakukan untuk mengetahui apakah penyu betina dewasa akan kembali ke pantai peneluranya untuk bertelur, skala geografis dati tiap individu populasi penyu hijau yang bertelur (*breeding population)* dan penyebaran berbagai populasi penyu hijau di kawasan Australasia (Bab 3). Hasil analisa ini sangat penting untuk memahami

bagaimana penyu hijau be-respons terhadap perubahan ketersedian tempat peneluran dari waktu ke waktu dan penentuan skala ruang yang tepat untuk upaya perlindungan dan pemantauan populasi penyu hijau. Hasil kajian variasi DNA mitochondrion (mtDNA) dari 714 samples yang diambil dari penyu hijau yang bertelur di Aru dan dari 26 pantai peneluran yang ada di kawasan Australasi menunjukan adanya 25 macam haplotype, dengan melakukan analisa distribusi frekuensi diketahui adanya 17 jenis stok (*Breeding stock*)yang secara genetis berbeda (menunjukan unit pengelolaan, atau *Management Unit*, MU). *Breeding stock* tersebut berasal baik dari satu lokasi peneluran (misalnya Aru) maumpun kumpulan beberapa lokasi peneluran yang terpisah secara geografis satu dengan lainnya sekauh lebih dari 500 kilometer (mis. di bagian utara *Great Barrier Reef*). Haplotype yang unik, C14, ditemukan dominan pada struktur genetika populasi penyu hijau yang bertelur di Aru dan hasil perbandingan dilakukan dengan populasi lain di kawasan Autralasia menunjukan populasi penyu hijau di Aru memiliki suatu unit manajemen yang berbeda dengan yang lainnya. Isolasi secara genetis yang efektif ini membuktikan bahwa penyu betina kembali bertelur di lokasi peneluran (*rookery*) yang sama. Hasil analisa phylogenetis terhadap 25 haplotype menunjukan adanya 5 rumpun/ kelompok (*clade*) yang menjelaskan adanya pemisahan/partisi *sequence divergence* ((Φ = 68.4) antara rookery yang berada di samudra Pasifik dan samudra Hindia (Kawasan Asia Tenggara). Perbedaan secara genetik ini disebabkan karena adanya penghalang daratan pemisah di antara australia bagian utara dan New Guinea (sekarang ini menjadi Torres Strait) yang terjadi pada masa Pleistocene. Walaupun bukaan terhadap penghalang daratan ini berulang terjadi setiap 100.000 tahun dan adanya kedekatan genetis diantara populasi penyu hijau di kawasan Great Barrier Reef, deviasi genetis tidak semata-mata menjadi pudar. Adanya *natal philopatry* pada skala subregional secara jelas menyediakan penyangga yang efektif untuk mencegah terjadi-nya proses homogenisasi secara menyeluruh di kawasan ini dalam kurun waktu yang sangat panjang.

Data genetika membuktikan bahwa aliran gen (*gen flow*) diantara populasi penyu hijau cenderung terjadi diantara rookery yang berjarak dekat (sejauh 500 km) dan jarang terjadi diantara rookery yang saling berjauhan letaknya. Adanya korelasi yang signifikan antara pemisahan secara genetic dan jarak antar *rookery* pada skala hingga 2000 km (*'isolation-by-distance'*). Namun demikian, korelasi ini agak lemah bila dibandingkan dengan jenis penyu lain yang memiliki kemampuan berpencar yang mirip dengan penyu hijau. Secara keselurahan, dinamika struktur stok dari populasi penyu hijau di kawasan Australasia dipengaruhi oleh kemampuan penyu betina untuk bergerak berpindah-pindah ke tempat perkembangbiakannya yang berdekatan. Struktur populasi yang disimpulkan melalui analisa mtDNA sejalan idengan skala pergerakan penyu yang diamati melalui studi mark-recapture

terhadap populasi penyu hijau di kawasan Australia Tinur yang dilakukan dalam jangka waktu yang panjang.

Penyu hijau (*C. mydas*) merupakan contoh klasik jenis satwa bermigrasi jauh, dimana penyu dewasanya melakukan migrasi antar habitat pakan dan habitat peneluran yang jaraknya bias mencapai ribuan kilometer. Jangkauan geografis dan arah pergerakan oseanik bervariasi untuk tiap populasi maupun individu dalam suatu populasi yang sama. Pada umumnya, kumpulan penyu yang berada dalam satu habitat pakan berasal dari beberapa populasi yang berbeda. Padang lamun di bagian timur kepulauan Aru merupakan didiami oleh populasi penyu hijau dalam jumlah yang sangat besar. Penemuan penyu yang ber-tanda (tagged turtles) yang berasal dari kawasan laut Sulu, Australia Barat dan bagian utara *Great barrier Reef* , menunjukan bahwa penyu yang bertelur di tempat tersebut bermigrasi ke kawasan kepulauan Aru sebagai habitat pakannya (Bab 4). Analisa secara sistematik tentang komposisi kumpulan/aggregasi penyu di habitat pakan di Aru menyimpulkan asal penyu di yang mendiami habitat ini yang mendapat dampak dari aktivitas eksploitasi yang dilakukan di kepulauan Aru. Analisa *mixed stock* dengan menggunakan berbagai haplotipe mtDNA yang teridentifikasi diantara populasi penyu yang bertelur, dipakai untuk menelusuri konektifitas migrasi di antara tujuh habitat pakan yang terletak sepanjang transek 5000 km di pesisir utara Australia dan 17 stok genetik di kawasan Indo-Pasifik. Hasil analisa mixed stock dari beberapa kumpulan populasi penyu di habitat pakan di kawasan Indo-pasifik menyimpulkan adanya suatu jaringan konektifitas yang kompleks di antara habitat pakan dan habitat peneluran di kawasan ini. Kebanyakan habitat peneluran menyokong lebih dari satu stok populasi.Habitat pakan di kepulauan Aru dan Bagian barat daya *Gulf of Carpentaria* (Australia Utara) dengan persentasi keterwakilan masing-masing; 49 ± 8.8% dan 93 ± 2.6%. Jarak geografis diantata habitat perkembang biakan dan habitat pakan sangat mempengaruhi keterwakilan suatu stok pada satu habitat pakan (w_i = 0.654) namun bukan merupakan ukuran untuk besarnya persentasi keterwakilan. Disamping stok Aru, stok dari Papua dan dari bagian nGBR juga terwakili dalam populasi penyu yang mendiami habitat pakan di Kepulauan Aru. Namun demikian, hasil assessment yang sama di habitat pakan yang lain tidak menunjukan adanya keterwakilan dari stok yang berasal dari Aru. Hal ini menunjukan bahwa penyu yang bertelur di kepulauan Aru tidak ber-migrasi ke arah selatan memasuki perairan Australia. Namun demikian, standard error yang relatif tinggi pada nilai estimasi kontribusi menyebabkan kesimpulan yang tempat sulit ditentukan. Hal ini sebagian besar disebabkan oleh kelemahan analisa yang berhubungan dengan adanyan jenis haplotipe yang dimiliki oleh berbagai populasi yang dianalisa, dan juga karena upaya pengambilan sample di beberapa lokasi kurang lengkap yang menyebabkan hasil analisa menjadi bias, sehingga tidak seluruhnya mampu mendeteksi kontribusi dari daerah peneluran yang jauh jaraknya.

Perbedaan kontribusi yang secara proporsional menunjukan bahwa dampak ekplotasi penyu secara local terhadap populasi penyu bertelur yang terpencil harus dikaji secara khusus.

C. mydas dieksplotasi karena daging dan cangkangnya diperdagangkan secara komersial maupun subsisten. Pengambilan langsung dan tidak sengaja melalui aktivitas perikanan merupakan sumber mortalitas di kwawasan Indo-Pasifik, dan merupakan penyebab menurunnya populasi baik local maupun regional secara drastic. Di kawasan Aru, pengambilan *C. mydas* terjadi baik di pantai peneluran maupun di habitat pakan penyu. Karena beragam stok populasi penyu mendiami habitat pakan di perairan Aru, maka dampak eksplotasi ini bersifat regional. Karena itu, walaupun secara global *C. mydas* masih bertahan karena kesuksesan upaya konservasi local, populasi penyu secara regional masih saja terancam. Viabilitas stok penyu yang bertelur di kepulauan Aru dan stok yang bermigrasi ke perairan Aru diteliti dengan menggunakan model berdasarkan umur (*age based model*) dengan masukan data genetik dan demografi (Bab 5). Model ini merupakan pendekatan yang handal untuk menentukan ketahanan sebuah stok populasi. Data untuk analisa ini berasal dari hasil penelitian di Aru (lihat Bab 2,3, dan 4) dan data hasil ekstrapolasi dari stok yang berasal dari kawasan nGBR. Istilah 'Stok Aru' pada bab ini menunjukan semua individu penyu yang kembali bertelur di Pulau Enu pada tiap siklus perkembang-biakan, sedangkan istilah 'populasi Aru merupakan semua individu yang bisa dipantau di sekitar peraitan kepulauan Aru sepanjang siklus hidupnya. Model ini memprediksikan bahwa baik stok penyu di Aru maupun populasi penyu di Aru dapat bertahan bila pengambilan langsung individu penyu dewasa berada di bawah jumlah 3000 ekor per tahun. Hal ini terjadi bila sumber mortalitas lain, baik antropogenik maupun secara alami diasumsikan tidak ada. Namun demikian, komposisi stok Aru maupun populasi Aru diduga dapat berubah: proporsi penyu yang bermigrasi dari kawasan yang jauh secara relative bertambah dibandingkan dengan individu yang melakukan aktivitas makan ataupun bertelur di Aru. Meningkatnya laju eksploitasi akan menyebabkan kemungkinan kepunahan penyu juga meningkat. Skenario terburuk yang dihasilkan dari analisa ini meliputi upaya eksploitasi penyu pada keseluruhan habitat pakan serta dan penyu betina dewasa dan telurnya di seluruh tempat peneluran di Aru. Hasil analisa dalam skenario terburuk ini memprediksikan bahwa stok penyu di Aru akan mencapai titik kepunahan dalam kurun waktu 50 tahun. Skenario in juga digunakan untuk mengkaji beberapa strategi pengelolaan yang mungkin dilakukan untuk mengurangi resiko terjadinya kepunahan pada stok penyu Aru. Bila intervensi pengelolaan hasil berfokus pada perlindungan sarang pada tempat peneluran di Pulau Enu maka akan berdampak kecil bagi pemulihan populasi. Jika tingkat kematian penyu betina di pantai peneluran di Pulau Enu menurun hingga nol, resiko kepunahan akan menjadi 50% dalam kurun waktu 200 tahun. Bila penetapan kuota penangkapan penyu sebesar 500 penyu dewasa per tahun (dari habitat pakan)

termasuk dalam kegiatan pengelolaan, maka kemungkinan kepunahan menjadi rendah. Jumlah ini cukup untuk memenuhi kebutuhan pasar lokal (subsisten). Penggunaan scenario pengelolaan yang sama pada habitat peneluran dan habitat pakan yang jauh tidak akan meningkatkan daya tahan stok penyu terhadap kepunahan.

Populasi penyu hijau di Indonesia tertekan dari eksploitasi komersial dan subsisten. Untuk menemukan praktek yang terbaik dan skenario manajemen pembiayaan yang efisien dan juga tanggung jawab kepemerintahan, setiap kasus perlu dinilai tersendiri, sambil mempertimbangkan karakter lintas batas (internasional dan nasional) sejarah hidup dan ancaman terhadap penyu laut (bab 6). Berbagai macam eksploitasi mempengaruhi penyu hijau di Aru dan dampak kolektif ekspoitasi tersebut terhadap kelangsungan populasi ini membutuhkan manajemen yang cukup baik dan intervensi dalam bentuk *monitoring*. Hasil keseluruhan yang di tulis di empat bab data dari tesis ini, menunjukan manajemen intervensi dilakukan paling baik di tingkat lokal di kepulauan Aru untuk menekan kemungkinan kepunahan, tanpa memerlukan skema co-manajemen internasional yang rumit. Penelitian ini menegaskan bahwa, di tingkat regional, konservasi *C. Mydas* harus dilakukan dengan pemahaman mendalam tentang distribusi stok, saling keterkaitan mereka dan dinamika populasi lokal. Strategi manajemen konservasi yang disarankan di bab 6, seharusnya menunjukkan pemahaman ini, teteapi juga mengakui kepedulian sosial yang menyertai konservasi keanekaragaman hayati.

Penelitian di masa depan

Masih ada ketidak pastian yang mempersulit proyeksi akurat populasi penyu hijau di Aru dan daerah-daerah lain di Indonesia. Sebagai contoh, tanpa estimasi *reliable* besarnya agregasi pencarian makanan, proporsi dari stok berbeda yang terlibat dalam wilayah makan tidak bisa di terjemahkan ke angka ril jumlah individu (1 - ß di dalam analisa viabilitas di diskusi dalam bab 5). Oleh karena itu, tetap tidak jelas apakah proporsi dari stok terpencil yang telah di observasi, yang terpengaruh eksploitasi, akan punya impak besar terhadap stok terpencil tersebut. Lebih jauh, stok bisa bertahan terhadap suatu ekploitasi tanpa menghadapi kepunahan tergantung ukuran dan perilaku migrasi stok itu (bab 5). Oleh karena itu, penelitian masa depan harus diarahkan pada pengumpulan jenis-jenis informasi seperti di atas, misalnya melalui analisa genetic yang diarahkan pada identifikasi perbedaan di antara penyu yang bermigrasi ke pantai-pantai terpencil untuk bertelur dan penyu yang tetap di wilayah makan di Aru.

Kemunduran populasi ditengarai terkait dengan perubahan kondisi habitat makan, pantai bertelur, dan wilayah migrasi. Sistem peringatan dini terkait perubahan populasi akan menjadi

perlu dalam perubahan tingkat bertelur, tingkat pertumbuhan, rasio jenis kelamin penyu muda, atau perubahan demografi di wilayah makan. Informasi yang disajikan di tesis ini, dilengkapi dengan data dari penelitian lain merupakan data dasar yang kuat terkait kerangka analisa viabilitas populasi (PVA) yang akan melengkapi proses spasial (penggunaan landscape), demografi, dan ekosistem (interaksi ekologi) untuk membuat perkiraan terbaik tentang dampak manusia (misal eksploitasi). Oleh karena itu, pengumpulan data dasar dan data eksperimental secara terus-menerus untuk semua spesies dan populasi terkait siklus reproduksi, system control fisiologis, dan parameter ekologi terkait menjadi sangat penting. Jika tidak, pada saat terjadi perubahan lingkungan yang sangat cepat, kita tidak akan mempunyai informasi penting yang diperlukan untuk menilai dampak yang mungkin dan kemungkinan terjadi pada populasi penyu laut dan menerapkan praktek manajemen dini dan tepat.

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There were many rows of giant turtles, front fins tied together above their heads with strings of raffia ribbon, hanging off bamboo poles awaiting to be sold for their meat. This was my first encounter with sea turtles, a six-year old at the hand of her father on Serangan Island, Bali. We were in Meru Betiri National Park on the south-eastern coast of Java when I first witnessed a turtle breaking the surface of the sea, moonlight reflecting off its wet carapace, slowly moving up the beach, labouring to dig a nest and deposit her eggs and finally moving back to the sea. Watching that process is an incredibly magical experience, now as much as it was then. Many years on, while diving off the east coast of Malaysia, I witnessed numerous large adult female green turtles resting on the seafloor during what I now know to be their 'internesting period' and watched hatchlings emerge from their nests at the hatchery on the beach of pulau Redang. These events set the course for my aspiration to study the ecology and conservation status of the green turtle in far South-eastern Indonesia, which has subsequently resulted in this thesis.

Without a doubt, the years of field surveys on Enu Island and south-eastern Aru have truly been life changing experience; living in isolation, under primitive circumstances (tent, no power, no fresh water, no furniture) for periods up to 5 months on end. 'Home' was a near-pristine beach that had been used as nesting ground by green turtles (and in the past by hawksbill turtles as well) for hundreds or probably thousands of years. Despite the hard labour of walking approximately 18 kilometres every night and again the next morning, tagging and measuring hundreds of turtles, the magic of watching these giant ancient creatures emerging from the sea and moving up a shiny white beach has never worn off. There are many people I wish to thank for making this experience such a memorable one.

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On January 14th 1999 Dobo was rocked by 4 days of rioting which ensued from the political instability that had held the country in its grip for months and had led to the fall of the Suharto regime. Barely a week later serious rioting broke out in Ambon which lasted for many years and left thousands of people dead, homeless and displaced. Under these circumstances, studying was impossible, let alone conducting field research and it marked the abrupt end of the work in Aru. With one more visit in 2000 and a short survey conducted by the local Aru-team, the survey work was largely left unfinished and I had to look for a new direction in the research project. The solution presented itself during a sea turtle symposium in Sabah, Malaysia. A group of researchers at the Zoology and Entomology department of the University of Queensland had been working on unravelling the phylogeography of the green turtle in Australian and neighbouring waters and were looking to find tissue samples from the Aru and East Kalimantan region. In June 2000 I started in the ZEN lab as a complete novice in the field of population and conservation genetics. This period was marked by a steep scientific learning curve and by the development of some fantastic lasting friendships.

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Government (DEST). Thus I commenced what ended up being a 2 ½ year collaboration with Nancy FitzSimmons in her Wildlife Genetics lab at the University of Canberra. Not only did Nancy supervise my research work into the genetic distribution of turtle across several feeding grounds but she employed me to work on other projects when the DEST funding had run out but my desire to remain in Australia had deepened. Again, I found myself amidst a fantastic group of people in the lab and office space and in particular I want to thank Lachie, Michelle, Dave, Alex, Ollie and Niccy for their friendship and support.

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Over the course of this long-term project I have lived in many places for shorter or longer periods of time – close to 25 addresses, with a maximum of 2.5 years in 1 place and an average survival rate of 6 months and 3 weeks in 1 place. I have only been able to survive this migratory lifestyle due to the generosity and companionship of many people with whom I shared a house. Here I particularly want to acknowledge Gilly for providing me with free accommodation, G&Ts, shared spas and gyms and many discussions on marine conservation in both Bali and Brisbane. While I have spent probably 90% of my time "overseas" in Indonesia and Australia, some friends "back" in the Netherlands and elsewhere have never ceased to support and encourage me to do and finalise this work. Thank you guys, without your friendship I would not be where I am now; Marieke, Astrid, Gertjan, Frederique, Irma, Janneke, Anneke, Ingvild.

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Curriculum Vitae

Kiki (Elisabeth Margaretha) Dethmers was born on 29th of June 1966 in Leiden, the Netherlands. In 1970 she moved to Semarang, Indonesia with her family and attended kindergarten and the first 2 years of primary school at local Indonesian schools. Subsequently, she received home schooling through the Dutch IVIO (Instituut Voor Individuele Ontwikkeling) system. After the sudden death of her father in 1976, she moved back to the Netherlands where she graduated from secondary school in 1984 (HAVO at the Christelijke Scholengemeenschap Zandvliet, Den Haag). The following year she volunteered at the reception of Chateau de Bossey, a conference centre of the World Council of Churches in Celigny, Switzerland. After completion of a one-year science study at Carleton University in Ottawa, Canada in 1986 she returned to the Netherlands and continued to study environmental science at the H.C. van Hall Institute in Groningen, from which she graduated in 1990. She worked for a forestry training project (at the Balai Latihan Kehutanan) and for Wetlands International (in those days the Asian Wetland Bureau) in Bogor, Indonesia, but decided to continue her education in environmental science. In 1994 she obtained an M.Sc. degree for Environmental Science at the Wageningen Agricultural University and in June 1996 she commenced a Ph.D. project on the ecology and phylogeography of green turtles in Indonesia through the Radboud University in Nijmegen. This project allowed her to move back to Indonesia in January 1997 and in the following 2 years she moved back and forth between Ambon and the Aru Archipelago until the political riots erupted in late January 1999. In 2000 she moved to Australia and worked in various conservation and evolutionary genetics laboratories at the Universities of Queensland and Canberra. Since June 2008 she is working on marine megafauna conservation and marine protected area planning projects in Timor Leste and the Northern Territory for the NT government. She remains actively involved in research and conservation programs on sea turtles in the Indo-Pacific region.

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